

ELECTROLYTIC DETERMINATION OF EXOSMOSIS FROM THE ROOTS OF PLANTS SUBJECTED TO THE ACTION OF VARIOUS AGENTS

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I. INTRODUCTION

In a previous paper the writer ('15) gave some results showing the exosmosis curves when normal growing plants are taken from a full nutrient medium and placed in redistilled water. Those results and the data herewith given show that exosmosis of electrolytes is a constant feature associated with the transfer of normal growing plants from a full nutrient solution to distilled water. In the paper above mentioned evidence was introduced indicating that such exosmosis was not a causal injury but that it was simply a concomitant condition or incidental effect and had but an indirect relation to the inimical condition of the plant in the distilled water. For convenience we might designate the agency or agencies causing such exosmosis as *passive* in their effects.

In this paper are given results on exosmosis in terms of the electrolytic conductivity of the medium when such excretion is caused, or at least is accelerated, by various factors or agencies which we may designate as *active* in their effects. Accordingly, plants have been treated by injurious agents or subjected to conditions of different kinds and the comparative effects on the exosmosis from the roots have been noted. By determining the conductivity of the medium at various intervals subsequent to the treatment, data have been secured for plotting the exosmosis curves shown in this paper. It has also been the aim to determine in each case the approximate boundary between the normal and the abnormal exosmosis by varying either the duration of application or the concentration of the substance applied, or both. Hence in most cases there will be found the two extremes with any given substance—at the upper end of the scale the curve of excessive exosmosis due to

cytolysis or death of the cells (though it should be noted here that excessive exosmosis from the roots may result even when those tissues are in an apparently normal condition), and at the lower end of the scale the curve of slight exosmosis that is in the region of the normal curve of exosmosis for untreated plants placed from full nutrient solution into distilled water. Between these two extremes lie various gradations depending on conditions.

II. HISTORICAL REVIEW

The work that has been done on the problem of excretions from the roots of plants is very interesting from several stand-points and has been considered by various workers to be of great practical importance. Nearly a century ago De Candolle ('32) advocated a theory of crop rotation on the basis of root excretions in which he claimed that certain plants excreted from their roots substances which are harmful to succeeding crops of closely related plants, but not so to plants less closely related. This theory was based partly on his own observations and partly on the statements of earlier workers.

At De Candolle's suggestion Macaire ('32) performed some experimental work pertaining to root excretions. He took plants from the soil, washed the roots carefully, and placed them in rain water. After several days, during which the water was frequently changed, the water was yellow and had odor, taste, and chemical reactions indicative of contained exuded materials. By placing one part of the roots of a plant in a vessel of pure water and another part in a second vessel containing a solution of lead acetate and later finding the salt in the pure water, he concluded that a plant can excrete a poison which it has absorbed. The results of Macaire's experiments with water cultures led him to favor the theory of crop rotation on the basis of the excretions from the roots of plants, as advanced by De Candolle.

Braconnot ('39) repeated many of Macaire's experiments but was unable to convince himself that plants excrete toxic substances from their roots, and hence he did not look with favor upon De Candolle's theory. Braconnot believed that

capillary action played a rôle in Macaire's experiments whereby he obtained an excretion of lead acetate into distilled water, as noted above. Boussingault ('45) considered that under ordinary conditions radicular excretion is doubtful, and that any excretion from the roots in water is caused by disease. He also advanced various arguments opposing De Candolle's theory.

Gyde ('47) grew various agricultural plants in soil for a time and then, after carefully washing the roots, placed them in pure water. After 3-17 days, during which the plants continued in good condition for the most part, the water was evaporated. The finding of a residue of yellowish or brown matter, part organic and part inorganic, caused him to conclude that plants excrete both organic and inorganic substances in minute quantities, similar in composition to the sap. But he denied that root excretions have any injurious effect upon plants later grown in the same medium.

An examination of the literature on the subject of root excretions reveals the tendency among the workers of the particular period at which we have now arrived in our review, to pay more attention to the morphological and chemical aspects of root excretions, and perhaps not so much to the purely agricultural phases. Hence we find from this period on, considerable emphasis laid on the structure of the root and a more detailed account given regarding the chemical nature of the substances excreted from the roots, even though the experimental methods were somewhat crude in most cases. Furthermore, it should be said that opinion was divided on the question of whether or not there is an actual excretion from the roots.

Among those whose influence was felt in the development of the chemical aspects of the subject at this time Liebig should probably be mentioned first. In the American edition of his work ('41, p. 195) occurs the following statement: "It is evident that plants, also, by producing carbonic acid during their decay, and by means of the acids which exude from their roots in the living state, contribute no less powerfully to destroy the coherence of rocks." An appended note by Dr. Webster in the

same work ('41, p. 411) says that other chemists were unable to obtain results similar to those of Macaire. If they did, they were inclined to ascribe them to injury of the roots examined.

Various workers were thus attacking different phases of the problem. Chatin ('47) mentioned the excretions from roots and especially considered the elimination of toxic substances by them. Link ('48) held that the slimy drops found on root tips should not be considered as actual excretion inasmuch as they arise from the cast-off cap cells of the root. Garreau and Brauwers ('58) maintained a similar view in regard to the gummy, nitrogenous substance they found given off by the roots to the water in which they were placed. The observations of Liebig ('58) concerning the dissolving action of roots on limestone were later substantiated by the experimental work of Sachs ('60), which has been so much referred to since that time. Of the two possible explanations Sachs advanced—excretion of carbonic acid by the roots, and the liberation of acids by the decomposition of the cell walls of the roots—he inclined to favor the latter as being the best explanation for the marble etchings caused by the roots in his experiments. In his extensive series of experiments, Knop ('60, '61, '62) studied, among other things, the character and amount of root excretions from certain plants placed in distilled water, and the conditions governing the same. His analyses indicated that, in addition to other substances in small amounts, potassium, calcium, phosphoric acid, and some organic matter were excreted. The studies of Cauvet ('61) resulted in his declaring that physiologically sound roots do not excrete any substances, toxic or otherwise, and that all theories based on the ideas of root excretion advanced by De Candolle and Macaire were necessarily false. Sachs ('65) made further contributions along his line of work indicated above, while Liebig ('65) says:

“Wir haben allen Grund zu glauben, dass diese Absonderung an der ganzen Oberfläche stattfindet, wir beobachten sie nicht nur am Stämme, sondern auch an den kleinsten Zweigen, und wir müssen daraus schliessen, dass dieser Excretionsprocess auch an den Wurzeln vor sich geht. . . . Eine Ausscheidung von Excrementen kann demnach bei den Pflanzen

nicht gelegnet werden, wiewohl es möglich ist, dass sie nicht bei allen Pflanzen in gleichem Grade stattfindet."

Molisch ('87) branched out in a new direction as regards the subject of root excretions; he held that such excretions exercise an influence on organic bodies in the soil which is even more important than that exercised upon the inorganic constituents of the same, for he considered the latter merely a dissolving action but the former a real chemical transformation. His main work along this line pertained to a study of the ferments in the root excretions, and their reactions and properties. Johnson ('90), after considering Gyde's results above noted, says that "we may well doubt whether agricultural plants in the healthy state excrete any solid or liquid matters whatever from their roots," but that "under certain circumstances, small quantities of soluble salts or free acids may indeed *diffuse* out of the root-cells into the water of the soil. This is, however, no physiological action, but a purely physical process." Goebel ('93) found that after the roots of *Hordeum* and *Lepidium* plants had been in distilled water for six days the medium gave the reaction for formic acid.

We thus see that the early work on root excretions was characterized by contradictions and uncertainties. While the nature of the more recent work has been more exact and comprehensive, the subject, as we shall see, is still beclouded by a considerable degree of confusion.

A classic piece of experimental work was undertaken by Czapek ('96, '96^a) to determine the exact chemical nature of the excreted substances from roots. In his report ('96^a) he discussed the earlier work, especially with regard to the relation between excretion from injured cells and actual exosmosis. In his experimental work he found that root excretions are composed of soluble substances, partly organic and partly inorganic. Of the inorganic, he identified K, Ca, Mg, HCl, H₂SO₄, and H₃PO₄, only the first and last mentioned—in the form of the primary potassium phosphate—being excreted in any quantity. Of the organic substances he identified carbonic acid and also formic acid, the latter in the form of its potassium salt; oxalic acid was also isolated as a primary

potassium salt. Czapek believes the reddening of litmus paper by root excretions to be due ordinarily to the acid reaction of monopotassium phosphate, but in the case of hyacinth roots to the primary oxalate. The corrosion of marble he attributed to the dissolving effect of carbonic acid. While considering as possible the results obtained by Molisch ('87), who claimed that diastatic ferments were normally present in the root excretions, Czapek's own work in repetition of Molisch's experiments offered only negative results.

Prianischnikov ('04) performed some experimental work dealing with the action of organic acids on phosphates. It will be remembered that because the roots did not attack aluminum phosphate Czapek concluded that organic acids were not excreted by them, inasmuch as this substance is soluble in certain organic acids. Prianischnikov found that phosphates derived from different sources were utilized by various plants but in different degree, and he suggested that this might be correlated with a different amount of CO_2 excretion, in which case the presence of organic acids would not be necessary.

Kunze ('06) found that free mineral acids are not excreted from the roots of higher plants and concluded that any acidity in the excretions is probably not due to the presence of acid salts of mineral acids, but to excreted organic acids. These, however, were present in such minute amounts as to be below the sensitiveness of litmus. He held that a greater effect is produced on the soil by fungi than by the roots of the higher plants. Lemmermann ('07) held views similar to those of Kunze.

Stoklasa and Ernest ('08) disagree with the findings of both Czapek and Kunze. No potassium or phosphoric acid were ever found as a result of their determinations, and they maintain that in the economy of the plant the excretion of such useful or necessary substances is unthinkable. Only CO_2 was found to be excreted under conditions of normal aerobic respiration of the root system; no other free inorganic or organic acids were detected. In aerobic respiration of the root system, they believe the organic acids in the living cells would be split up to give CO_2 and H_2 , the latter then being oxidized to H_2O .

They determined the amount of CO_2 excretion per gram dry weight of roots of wheat, oats, rye, and barley. The amount varied for the different plants but a correlation was found between the amounts of P_2O_5 , K, and Na contained in the dry roots of plants grown on gneiss and basalt and the amount of CO_2 excreted.

We now come to the work of various soil investigators whose results have again focused attention during the past decade upon De Candolle's original theory. The essential features of this work have become so well known that for our purpose it is not necessary to do much more than merely mention it here. Though not considering directly the phases of the subject with which we are dealing, yet the much-discussed paper by Whitney and Cameron ('03) is historically important and bears an intimate relation to the later work of the investigators in the Bureau of Soils of the U. S. Department of Agriculture, the results of which led to the so-called toxic-excretion theory. Among the workers most prominently connected with the early studies along this line may be mentioned Livingston, Britton, and Reid ('05); Livingston, Jensen, Breazeale, Pember, and Skinner ('07); Schreiner and Reed ('07); Schreiner, Reed, and Skinner ('07); Schreiner and Reed ('07^a); and others. As is well known, opinion is much divided on the various phases of this subject, however. Among those opposing the ideas or theories advanced along this line by the investigators named above should be mentioned Hopkins ('10); Hall, Brenchley, and Underwood ('14); and others.

That the question is one upon which investigations are still being pursued is shown by the publications from various quarters. As recent examples of these the work of Molliard ('13) and Prianischnikov ('14) may be cited. The former found that peas grown in water cultures in which previous crops of peas had grown produced a smaller growth than the original crops. This he attributed to the excretion of toxic substances in the medium by the earlier plants. The latter, from his own experimental work and from the results observed by him at the Rothamsted Experiment Station, is inclined to believe that the hypothesis of root excretion is not sufficiently demon-

strated. He says that other factors, as, for example, the physical nature of the soil, decomposition of roots, change in reaction of soil, etc., might be supposed to accomplish the same results as toxic excretions from the roots. In pure distilled water he found no decrease in either the size or quality of the crops of the second and third plantings, either where wheat followed wheat or where wheat followed oats. Experiments in sand, however, showed great decrease in the amount of the harvest of the second and third crops, but this, he believes, might be explained by the operation of the above-named factors.

So much for root excretions; we now come to a general consideration of exosmosis from living cells, both under natural conditions and under treatment of different kinds. While a great deal of attention has been given in the past to the intake, or endosmosis, of substances by the cell from its surrounding medium, comparatively little has been done on the opposite effect—the outgo, or exosmosis, of substances from the cell. It should be said, however, that the latter process, both in extent and in importance, is no doubt of much less significance in the plant's economy than the former.

Sachs ('60^a) referred to the exosmosis of soluble material from germinating seeds when they remain for some time in distilled water. Knop ('64), in his studies on the absorption of salts by healthy seeds, also determined the quantities of the different salts which pass out of the seeds during the time they are swelling in distilled water. He found that both organic and inorganic substances were excreted. Hofmeister ('67) ascertained that when fresh pieces of sugar-containing plants were placed in water, no sugar passed out of the tissues into the medium. The much-cited experiments of De Vries ('71) showed that pieces of red beet placed in water for 15 days gave no trace of sugar or of colored material to the water during that time. In a NaCl solution of sufficient concentration, however, he obtained an exosmosis of both sugar and colored material. Turnips, beets, and the seedling roots of wheat, barley, and corn were used in the experiments of Boussingault ('74) but from none of them did he detect any

exosmosis of sugar into the water in which they were placed. Pfeffer ('76, '77) and Detmer ('79) also confirmed the results above noted regarding the absence of sugar in the water in which roots or other plant parts had been exposed for some time. Wilson ('81) found that in some cases (*Dionaea* and *Drosera*) the excretions may be influenced by external factors, e. g., partly by irritation caused by nitrogenous substances and partly by osmotic action. In general, he believed that the excretion of nectar is caused by the osmotic action of a fluid on the surface of the nectary. Pfeffer ('86) studied the effects of various organic acids (citric, picric, and tannic) and some inorganic compounds in causing the exosmosis of absorbed methylene blue from *Lemna*, *Trianea*, *Azolla*, and *Elodea*.

Wächter ('05) obtained considerable exosmosis of sugar, especially in the case of *Allium Cepa*; he found, however, that salts like NaCl and KCl tended to inhibit this exosmosis. He also investigated the effect of ether on this phenomenon. While he obtained greater exosmosis of sugar the first two days in a solution of ether alone than in one of ether and KCl, he attributed this increase to leaching from cells killed as a result of contact with ether, and believed that the ether itself has no effect on the actual process of exosmosis.

Lepeschkin ('06), from his experimental work on sporangia of *Pilobolus*, concluded that the exosmosis of water was due to an alteration of the plasma membrane caused by the anesthetics he used, provided the amounts employed were sufficient to be toxic. Small amounts of ether and chloroform, on the other hand, were found to decrease the exudation of water, and he believed this to be due to a decrease in permeability of the plasma membrane.

An interesting line of investigation was undertaken by Czapek ('10, '10^a, '10^b, '11) a few years ago to determine the surface tension relations of the plasma membrane. That work is especially pertinent to our discussion here because of the prominent part exosmosis played in his experiments. He used for the most part species of *Echeveria*, *Spirogyra*, and *Saxifraga*, in the cells of which is found a tannoid substance,

anthocyan, which is precipitated by caffeine, giving a loose compound of tannin and caffeine, called a "myelin-formation." Ammonia also gives this precipitate even in a solution as dilute as 1-15,000. Czapek investigated the effect produced by the application of a great variety of organic compounds and some inorganic acids in varying dilutions and for different periods of time, and determined the concentration at which exosmosis just occurred, i. e., the critical point. At the higher concentrations exosmosis of the tannoid substance readily occurred, as shown by the absence of the "myelin-formation" when caffeine or ammonia was subsequently added. At the lower concentrations exosmosis did not occur and a precipitate was obtained, while at the critical point the precipitate was barely visible and usually in the form of fine particles.

By the use of his "capillar-manometer," Czapek was able to measure the surface tension exerted by the various concentrations, and found that, considering the surface tension of water as unity, that of the critical concentrations was approximately .68 in most cases. This lowering of the surface tension he considered as essentially a physical phenomenon which is intimately connected with the osmotic activities of the plasma membrane and is to be differentiated from the toxic action of injurious substances, e. g., anesthetics, whose action is chemical in large part, since even in very dilute solutions these caused marked exosmosis. Czapek used both aqueous and colloidal solutions and found that in general the critical concentrations had a surface tension of .68 in terms of water as unity. Inversely, he therefore concluded that the surface tension of the plasma membrane was also approximately .68 for the plant cells investigated. In his study of acids he found results coincident with those of Kahlenberg and True ('96) in that N/6400 was the critical concentration for exosmosis of the tannin bodies, just as those workers had found it to be the critical concentration for growth of *Lupinus* seedlings in solution culture.

In his later experimental work Lepeschkin ('11) obtained additional evidence tending to confirm and add to his previous results, as mentioned above. Thus he found that aniline dyes

penetrated cells of *Spirogyra* more slowly in the presence of one per cent chloroform than when the anesthetic was not used. If the cells were killed by the narcotic the rate was the same as for normal cells. He also used *Tradescantia discolor* and by the plasmolytic method found that the permeability to KNO_3 decreased during narcosis. This he explained on the assumption that the anesthetics (chloroform and ether) accumulated in the disperse phase of the plasma membrane which thereby leads to a hindrance of the solubility of KNO_3 and aniline dyes in the same. He considered that his results therefore showed that Nathansohn's hypothesis regarding the mosaic structure of the plasma membrane is not correct.

Another important piece of work dealing with the phenomenon of exosmosis from living tissue is that accomplished by Lillie ('09, '10, '11, '12, '12^a, '13, '13^a, '13^b) and discussed at length in his various papers. Among other things he worked on the larvae of *Arenicola* and the eggs of *Arbacia*, each of which contains a pigment, and found that on placing them in NaCl or KCl solution (.55m) isotonic with sea-water, there was a rapid exosmosis of the contained pigment into the surrounding medium. When, however, the organisms were placed in the salt solutions to which had previously been added in a certain concentration any one of several anesthetics belonging to various classes (alcohols, esters, hydrocarbons, and miscellaneous compounds) a checking or possibly a complete prevention of exosmosis resulted. In general, all the anesthetics tried gave cytolysis in strong concentrations and therefore a rapid exosmosis of the pigment, while in weaker concentrations they showed a definite protective or anticytolytic action against the salt solution when used in conjunction with it. Lillie finds the explanation of the observed phenomenon in the relations of the plasma membrane, the salt solutions used having a permeability-increasing action which is offset or prevented by the temporary alteration of the membrane as the result of the action of the anesthetic. The alteration, he believes, is accompanied by an increase in the volume of the lipoid particles of the membrane.

In connection with the general subject of exosmosis it might

be well briefly to mention the results obtained by some of the earlier investigators working on the products excreted by the leaves of plants. De Saussure (1804) found that leaves immersed in distilled water soon lose a considerable amount of substance, composed for the most part of alkaline salts. Treviranus ('38) mentioned the results of various workers who studied the incrustation of minerals on the surface of leaves and found it to consist of calcium and silicon salts, especially of calcium carbonate. Gaudichaud ('48) and Payen ('48) both found that there is an alkaline excretion on certain parts of the leaves of some plants, yet they disagreed as to the extent of this phenomenon in nature. Sachs ('62) ascertained that drops of water on the leaves of certain plants soon become alkaline, which he considered to be the result of an outward diffusion of alkaline salts in the leaf. Volkens ('84) studied the deposit of calcium carbonate found on the leaves of various plants. Dandeno ('02) made a comprehensive study of the different phases of the subject. Among other things, he determined that the alkaline substances extracted from leaves by distilled water are largely potassium and calcium carbonates and probably potassium oxalate. He further found that the residue from the evaporation of dew drops, guttation drops, and of water used in drenching the leaves is practically the same, and is similar to the calcareous deposit found upon the leaves of certain plants. The above investigations may therefore be considered as tending to substantiate the idea of exosmosis from leaves.

III. METHODS OF EXPERIMENTATION

The methods used for the electrolytic determination of exosmosis were the same as those described in the writer's paper referred to above. In that contribution (Merrill, '15) some of the curves were plotted on the basis of the specific conductivity. In the present paper, however, all curves are plotted on the basis of the values of x on the Wheatstone bridge when the resistance in the box is 9,110 ohms; as these values increase the specific conductivity also increases. In order to have a basis of comparison between the values of x

and the specific conductivity, the corresponding values of the latter for the values of x at 5, 10, 25, 50, 75, and 85 are given herewith:

Values of x on Wheatstone bridge for resistance of 9,110 ohms.	Corresponding values in terms of specific conductivity (to be multiplied by 10^{-5})
5.....	.23
10.....	.49
25.....	1.49
50.....	4.48
75.....	13.46
85.....	25.43

It is also advisable to have the conductivity values represented in terms of the concentration of some salt. The following are the values of the specific conductivity of NaCl solutions at 25°C. which had been determined by the writer for the concentrations indicated:

Concentration of NaCl	Specific conductivity (to be multiplied by 10^{-5})
N/16	686.13
N/32	353.94
N/64	181.93
N/128	93.25
N/256	47.79
N/512	24.54
N/1024	12.60

The correction for the specific conductivity of the water itself is not considered in the above values. Neither is that correction applied in any of the work here reported, since it is always a constant factor and only relative values are desired for the most part.

Plants of *Pisum sativum* were used. For the method of growing the seedlings, and other manipulations, see the writer's paper referred to (Merrill, '15). The plants were grown in full nutrient solution until a vigorous or well-developed condition was attained and then they were transferred to redistilled water¹ after rinsing the roots carefully and thoroughly in once-distilled water. Ten plants were grown in each culture. The treatment was always given when the plants were either in distilled water or in the solution, the effects of which on the plants were being studied. In all cases where the read-

¹ Hereafter, throughout this paper, whenever "distilled water" is referred to it will be understood to mean redistilled water with a specific conductivity of approximately 2×10^{-6} . If the ordinary distilled water is referred to, it will be specially designated as "once-distilled water" or some such distinguishing term.

ings were made in the distilled water, the resistance in the resistance box was 9,110 ohms. In some media other resistances were used; in such cases the values are given only in tables, and in terms of specific conductivity.

TABLE I
EFFECTS OF VARIOUSLY TREATED PLANTS ON THE DISTILLED WATER MEDIUM
AS SHOWN BY GROWTH OF SECOND CROP

Culture no.	Kind and duration of treatment	Green wt. of tops of 2nd crop* grams
1 and 2	Controls—no treatment; full nutrient to dist. H ₂ O	2.90
3	Plant tops packed in ice 19 hrs.; dist. H ₂ O unchanged }	2.80
4	Plant tops packed in ice 19 hrs.; dist. H ₂ O changed .. }	
5	In gas incubator at 50°C., 3.5 hrs.....	1.60
6	In gas incubator at 50°C., 3.5 hrs.....	2.55
7 and 8	Inoculated with <i>Ascochyta Pisi</i>	2.80
9	Illum. gas under bell jar, 6 hrs.; dist. H ₂ O unchanged...	2.75
10	Illum. gas under bell jar, 6 hrs.; dist. H ₂ O changed.....	2.25
11 and 12	N/1 MgCl ₂ in full nutrient as the solvent, 7 hrs.....	5.20
13 and 14	.5% H ₂ SO ₄ in full nutrient as the solvent, 7 hrs.....	2.30
15 and 16	1% KOH in full nutrient as the solvent, 7 hrs.....	8.45
17 and 18	Plants grown throughout in dist. H ₂ O; replaced by fresh seedlings in the unrenewed dist. H ₂ O.....	2.55
19 and 20	Same as Nos. 17 and 18, except that second crop was horse beans.....	6.85
21 and 22	Canada field peas in fresh dist. H ₂ O; no second crop ...	2.82
23 and 24	Horse beans in fresh dist. H ₂ O; no second crop	8.15

* The 2nd crop was 27 days old at time of weighing.

IV. PRELIMINARY EXPERIMENTS

In order to determine in a preliminary way whether the exosmosis from the roots of plants seriously affected by injurious agencies was sufficient to noticeably influence a new crop of seedlings in that medium (distilled water plus the excreted substances) as compared with control cultures in pure distilled water, the following series was set up. Canada field pea seedlings were grown in full nutrient solution until they were 15 days old, at which time they were about 8 inches high, and were green, vigorous, healthy, and in good condition. They were then treated in accordance with the plan given in table I. In some cases, depending on the nature of the agent applied, the treatment was given after the plants had been transferred to distilled water. This was the case with Nos. 3, 4, 5, 6, 9 and 10. Cultures 7, 8, and 11-16 were treated while still in the

full nutrient medium, after which they were transferred to distilled water. In all instances, however, the roots were carefully rinsed before being placed in the water.¹

To determine if any impurities had contaminated the distilled water in the cultures treated with ice, the distilled water in No. 4 was renewed after the operation. The resulting crop, however, was practically the same in cultures 3 and 4 and hence it may be considered that no plant food had entered from the ice. The distilled water was renewed in No. 10 a few hours after the treatment. The result of so doing was to discard the plant foods already excreted during, and immediately after, the treatment. This fact was evident from the better growth of the plants in No. 9 as compared with those in No. 10. Later work also showed that exosmosis caused by treatment with illuminating gas and other agents is comparatively rapid and immediate.

After the treatment the plants remained in the distilled water for 5-6 days, after which they were discarded. The distilled water level was then raised to the original height by adding fresh distilled water, and into this medium fresh Canada field pea seedlings were placed and the resulting growth determined. Cultures Nos. 17-24 are given in table I for comparison. After pea seedlings had been grown for 21 days in the unrenewed distilled water of cultures 17-20, the original plants were discarded and fresh seedlings of peas and horse beans were placed in the same distilled water. For comparison, cultures of these plants (Nos. 21-24) were set up at the same time in fresh distilled water.

Returning now to the effects of the treatments on the plants and noting the results given in table I, we see marked differences evident. Neither the ice nor the inoculation with *Ascochyta Pisi*² produced any effect either on the plants or on the excretions from their roots, and hence these cultures are sim-

¹ The usual method of rinsing throughout this work was as follows: The solution to be discarded was thrown out, the tumbler filled twice with once-distilled water (the roots replaced and the whole thoroughly shaken each time), and then distilled water (redistilled) was added, the roots replaced, and the readings taken.

² Cultures of *Ascochyta Pisi* were kindly supplied the writer by Dr. R. E. Vaughan.

ilar and comparable to the untreated controls. Marked injury resulted in the case of the heat, illuminating gas, $MgCl_2$, and H_2SO_4 , all in characteristic manner. The injury from KOH was rather slow in manifesting itself, but the coloration of the roots was a noticeable feature. An interesting condition to be noted here, which holds true also in the later experiments, is in regard to the effect of the heat and the illuminating gas. It should be borne in mind that during these treatments the roots remained in water. The tops only were affected and died; the roots remained white, turgid, and normal in appearance even though the exosmosis from them had been excessive, thus indicating a transfer of some electrolytes from the tops and down into the medium through the roots. Later experiments also substantiated the fact that abundant exosmosis sometimes occurs from roots which remain normal in appearance. The other agents ($MgCl_2$, H_2SO_4 , and KOH) caused more or less injury to both tops and roots. The exosmosis of nutrients into the water from the affected plants is evident by the greater growth of fresh pea seedlings placed in such water as compared with the controls. Both peas and horse beans grew somewhat better in fresh distilled water than in distilled water in which pea seedlings had already been grown for 21 days.

Further preliminary experiments along this line gave similar results. Thus in another series, vigorous, thrifty plants of Canada field peas grown 10 days in full nutrient solution were transferred, after rinsing the roots, to distilled water, some being untreated and others treated. The treatment consisted in placing some of the cultures in an atmosphere of illuminating gas for 3 and 6 hours, and others in a gas-heated oven for 1 and 2 hours where it was not aimed to keep the temperature constant. For those cultures in the oven 1 hour the temperature at the outset was $53^{\circ}C.$ and at the end $33^{\circ}C.$, while for those remaining in the oven 2 hours the initial temperature was $60^{\circ}C.$ and the final $33^{\circ}C.$ The conductivity of the water was measured soon after the plants were placed in it but before the treatment, and again 5 days after treatment,

at which time the plants were discarded and a fresh lot of pea seedlings substituted.

The average reading (value of x) of the water for the 4 untreated controls at the beginning was 14.6 on the Wheatstone bridge, and with the same resistance in the box (9,110 ohms) it was 12.6 after 5 days. For the 4 cultures treated with illuminating gas (2 cultures for 3 hours and 2 cultures for 6 hours, the resultant effect being approximately the same for the two periods of exposure) the average initial reading was 17.0 for a resistance of 9,110 ohms, and at the end of 5 days it was 43.7 for a resistance of 1,000 ohms. In this case the increase in terms of specific conductivity was from 9.2×10^{-6} to 317.3×10^{-6} .

In the 4 cultures placed in the oven at the temperature designated there was no marked difference as regards variation in conductivity of the medium. The average initial reading was 17.2 and at the end (after 5 days) it was 12.4, the resistance in the box being 9,110 ohms in both readings. The rather high initial readings in the above cases are due to the fact that it was some hours after the roots were placed in the water before the readings were taken. In later work it was found to be advantageous to have the interval between placing the roots in the water and taking the first reading reduced to exactly one-half hour in order to obtain comparative data on the initial rate of exosmosis under different conditions.

We have, of course, no indication from the above regarding the exosmosis or conductivity curve during the 5-day interval. Subsequent work shows that it is very probable that the curve rose considerably in the case of the untreated and the oven-treated cultures and then fell, at the end of 5 days, to a position lower than that of the initial reading, due to the absorption being greater than the excretion after the first 2 or 3 days.

Let us turn now to the results obtained with the fresh seedlings grown in the same water in which the first crop had remained for 5 days under the conditions indicated above. After the second crop had been growing in this medium for just 15 days the green weight of tops of the 4 cultures in each group

was determined, with the following results, the figures representing the average green weight of tops in each culture:

Previous crop untreated.....	3.39 grams
Previous crop treated with illuminating gas.....	4.38 grams
Previous crop in oven 1 and 2 hrs. at 60-33°C.....	3.35 grams

V. EFFECTS OF ANESTHETIC VAPORS

For this work the method used was to place the cultures (in some cases the medium also, in which instances the roots were in the water during exposure, and in other cases only the plants themselves, thus exposing the roots directly to the vapor) under bell jars into which the anesthetics were subsequently placed. In the case of ether and chloroform a meas-

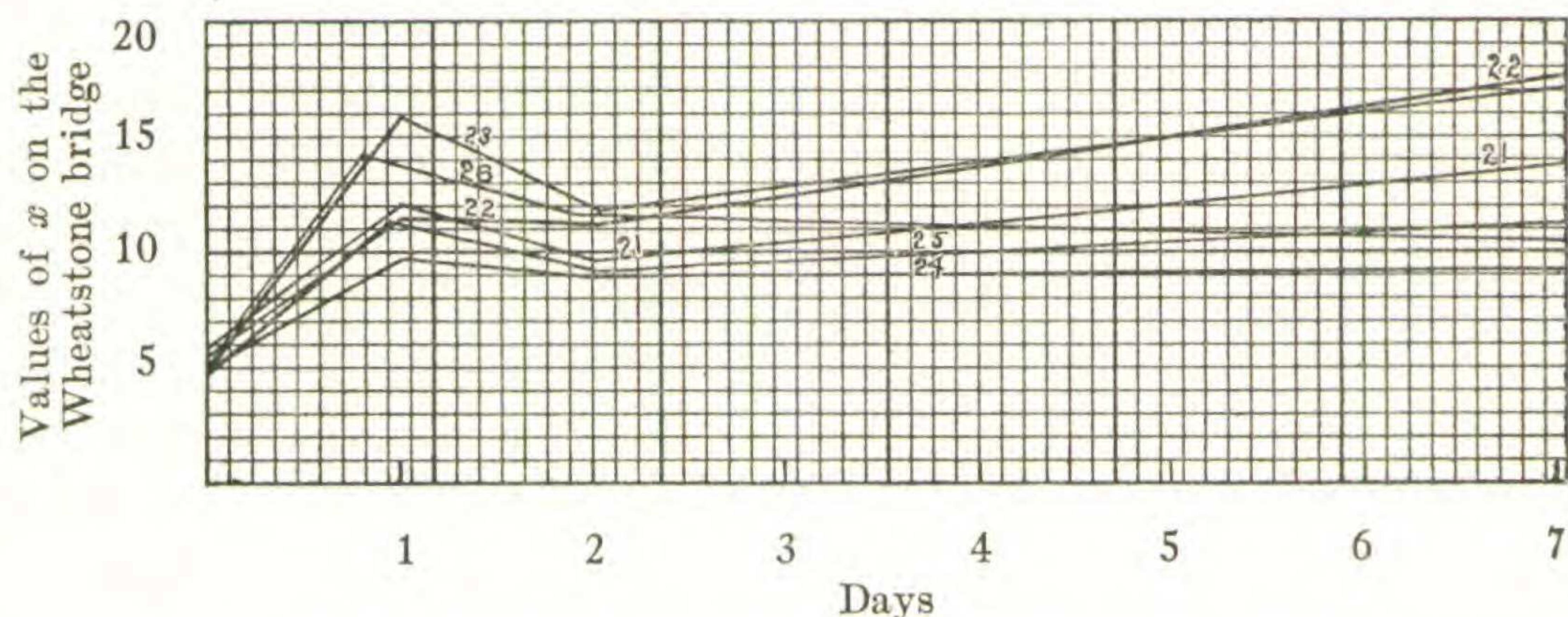


Fig. 1. Conductivity curves of cultures (series 11) in distilled water subsequent to treatment with anesthetics, as follows: No. 21, ether vapor, 1 minute, roots exposed; No. 22, control—roots exposed under bell jar 1 minute; No. 23, ether vapor, 2 minutes, roots exposed; No. 24, ether vapor, 5 minutes, roots exposed; No. 25, ether vapor, 10 minutes, roots exposed; No. 26, ether vapor, 15 minutes, roots exposed. The plants used were 39 days old. The first reading in each case is of the distilled water before the roots were placed in it.

ured amount of these agents was placed in an open evaporating dish under the bell jar, and after the treatment the residue was measured to determine the amount which had evaporated; in the case of the illuminating gas, however, the agent was run in until the air in the bell jar was more or less completely replaced. Where the plants alone were placed under the bell jars they were carefully attached by cheese-cloth bands to the leg of an inverted tripod, over which the bell jar was then placed.

In figures 1 and 2 are shown the results of treatment with ether and illuminating gas for varying periods of time. The plants for this experiment were 39 days old at time of treatment and had roots in good condition and well developed. The

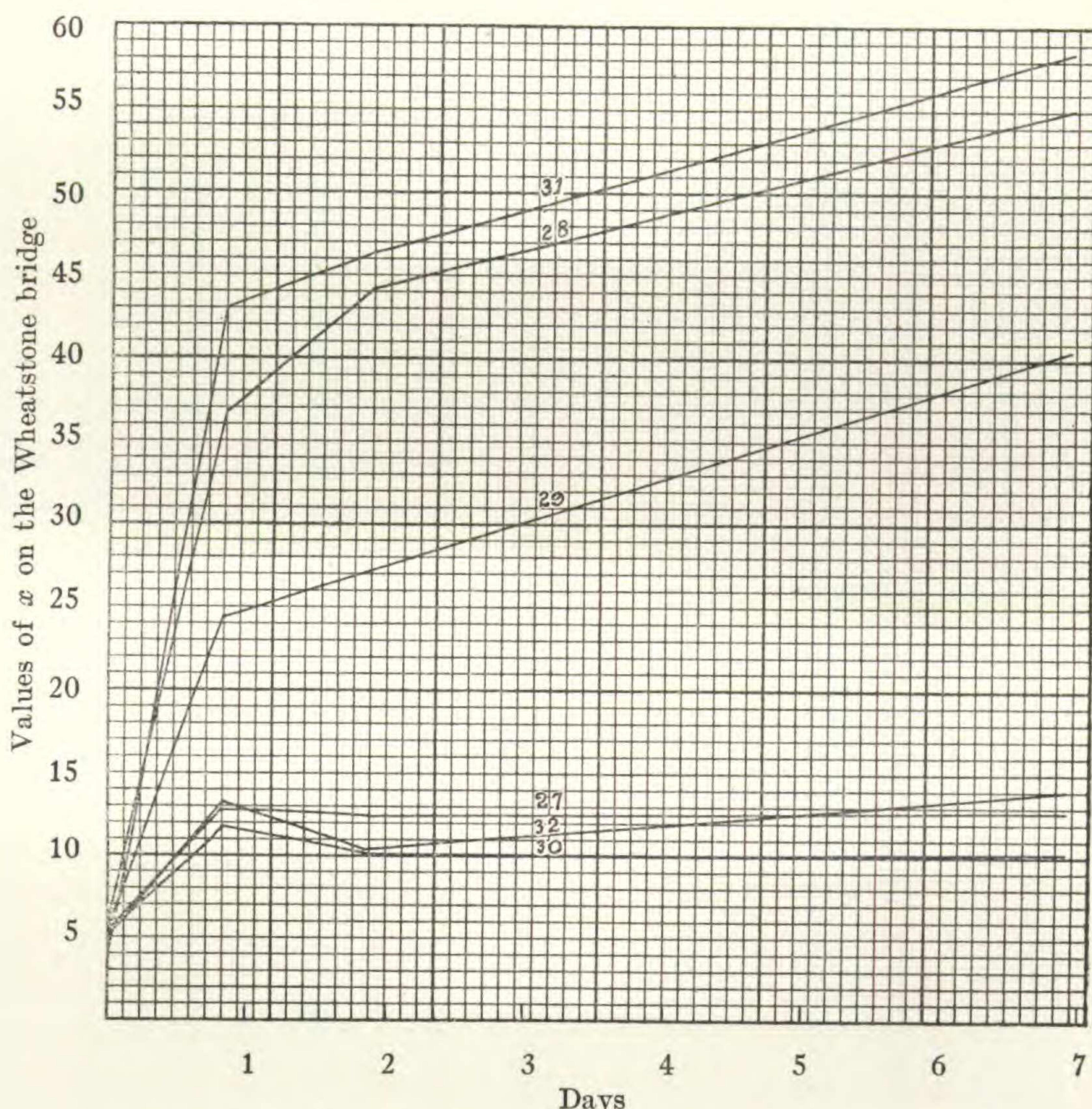


Fig. 2. Conductivity curves of cultures (series 11) in distilled water subsequent to treatment with anesthetics, as follows: No. 27, illuminating gas, 5 minutes, roots exposed; No. 28, illuminating gas, 10 minutes, roots exposed; No. 29, illuminating gas, 15 minutes, roots exposed; No. 30, control—roots exposed under bell jar 15 minutes; No. 31, ether vapor, 3 hours, roots in tumbler; No. 32, control—under bell jar 3 hours, roots in tumbler. The plants used were 39 days old. The first reading in each case is of the distilled water before the roots were placed in it.

first conductivity readings of the water were taken before the plant roots were introduced. As seen from the plotted results the ether had no effect on the exosmosis when the duration of the exposure ranged from 1 to 15 minutes; after 3

hours exposure, however, the exosmosis was pronounced, even when the roots were not in direct contact with the vapor.

An exposure of only 5 minutes to illuminating gas produced no effect, but one of 10 or 15 minutes' duration caused considerable exosmosis. That the 15-minute exposure should result in less exosmosis than the 10-minute one is an interesting point which finds an analogy, we shall see, at different places throughout the work, where in isolated cases a briefer exposure or milder treatment results in greater conductivity of the medium than a somewhat more prolonged exposure or more severe treatment. Where such a condition exists it is usually found near the boundary line of noticeable effect, and not where the effect is either nil or very pronounced. At this critical point the individual hardihood of the plants themselves seems the most plausible explanation of the difference. As the manipulation methods were exactly similar for any given series it is altogether unlikely that difference in technique was responsible for the variation.

The only plants to sustain any injury were those of cultures 28, 29, and 31. The tops of those in No. 31 drooped immediately after the treatment and soon died, though the leaves remained green; the roots, however, remained entirely normal to all appearances and retained their turgor. This is an interesting point and was referred to above. After 7 days Nos. 28 and 29 plainly showed some injury, but it was slight, and its visible effects were slow in making their appearance. At that time the tops of these cultures showed greater yellowing and drying than did those in the controls, No. 29 being somewhat more affected than No. 28; the roots of both, however, remained normal in appearance.

The greatest contrast between the treated plants and the controls is seen in fig. 3. The effect on the treated cultures corresponds to the duration of treatment, the curves especially showing the difference in the speed of initial exosmosis. It will be seen that the conductivity curves of the controls rise rather high during the first day. This is no doubt due to the effect of rather prolonged exposure of the roots to the air in the bell jar, even though it was saturated with water vapor.



Fig. 3. Conductivity curves of cultures (series 11) in distilled water subsequent to treatment, as follows: No. 33, illuminating gas, 15 minutes, roots exposed; No. 34, control—roots exposed under bell jar 15 minutes; No. 35, illuminating gas, 30 minutes, roots exposed; No. 36, control—roots exposed under bell jar 30 minutes; No. 37, illuminating gas, 1 hour, roots exposed; No. 38, control—roots exposed under bell jar 1 hour. The plants were 22 days old when treated. The first readings were taken in the various cultures after the roots had been in distilled water for the following periods: No. 33, 35 minutes; No. 34, 1 hour and 15 minutes; No. 35, 1 hour and 13 minutes; No. 36, 1 hour and 25 minutes; No. 37, 1 hour and 5 minutes; No. 38, 1 hour and 16 minutes.

The subsequent decline in the curve, however, is characteristic for normal root tissues. It is also seen here that the 15-minute exposure to illuminating gas resulted in a greater rise in the conductivity curve than did a similar exposure in the case of the cultures recorded in fig. 2. That this is due to the different ages of the plants in the two cultures was borne out by treatment of plants of different ages with other agents. The older the tissues the more resistant they become to the toxic substance. McCool ('13) was the first to point this out, in his experiments with manganese chloride, and we see that it here holds for anesthetics as well.

Figure 4 shows the effect of illuminating gas at different intervals when only the tops are exposed directly to the gas, the roots meanwhile remaining in distilled water. The plants were affected in proportion to the duration of treatment. The tops of No. 39 were only very slightly injured, so that there was practically no difference between them and the tops of the controls; No. 41 was affected more; and No. 43 still more, finally dying, after progressive drooping and yellowing. But here again the roots of the treated plants were in all respects similar to those of the controls and entirely unaffected, visibly, even though exosmosis was considerable. In such cases it was also presumed that the excreted substances came in part from the tops and that here we had an illustration of the downward flow of food materials which occurs in plants under natural conditions. This presumption was considered experimentally as follows:

Some cultures were placed under a bell jar and treated with illuminating gas as before, the roots meanwhile being in distilled water. The tops of one culture were not cut off, while those of another were removed just before treatment, and finally those of a third were removed just after treatment. The controls were not treated, but their tops were cut off immediately after the roots were placed in the distilled water. The treated plants all gave approximately the same exosmosis, which was considerably more than that from the controls. A point to be noted here is that even though the treated tops which were not cut off were very much affected,

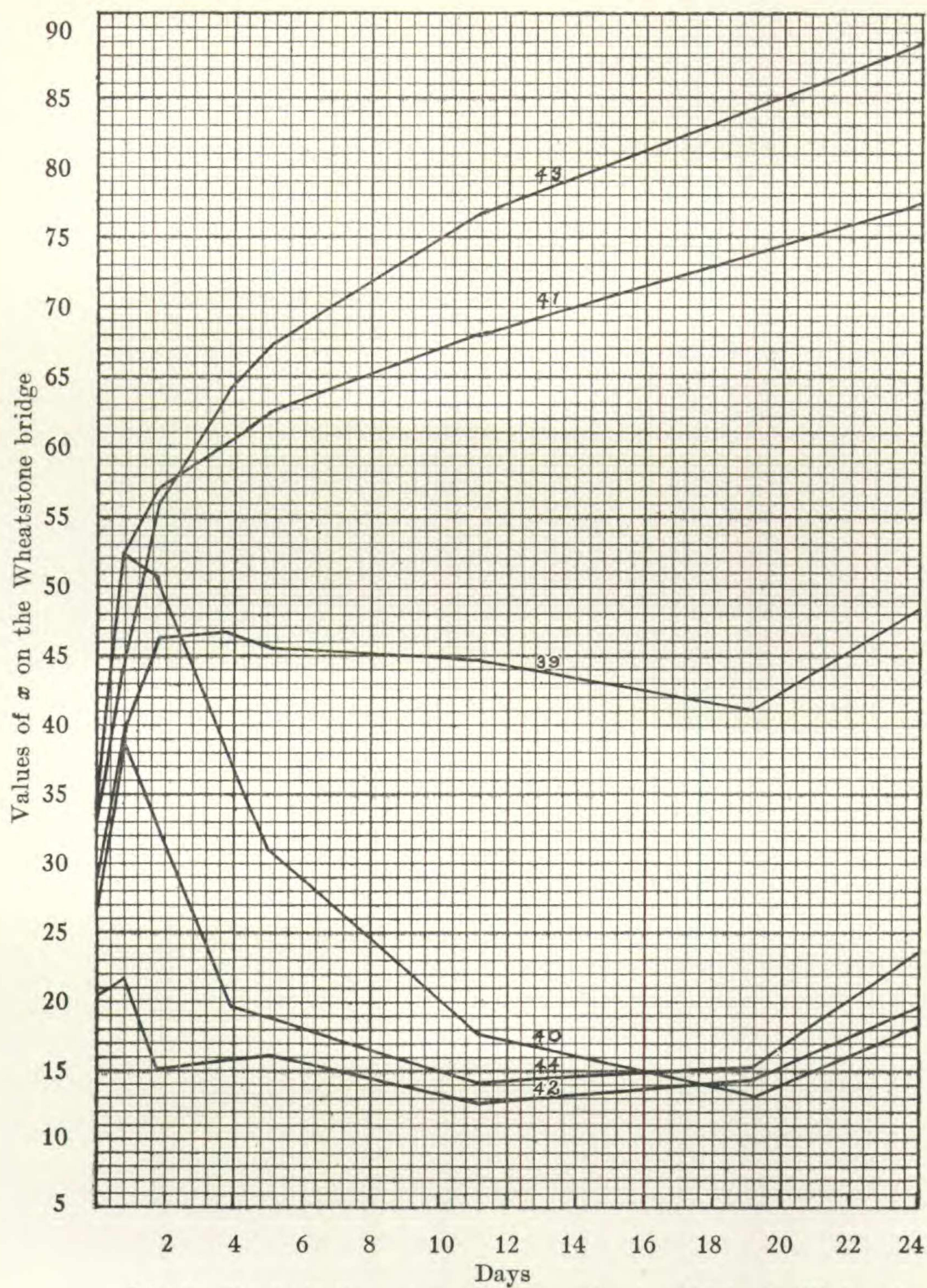


Fig. 4. Conductivity curves of cultures (series 11) in distilled water subsequent to treatment, as follows: No. 39, illuminating gas, 15 minutes, roots in tumbler; No. 40, control—under bell jar 15 minutes, roots in tumbler; No. 41, illuminating gas, 30 minutes, roots in tumbler; No. 42, control—under bell jar 30 minutes, roots in tumbler; No. 43, illuminating gas, 1 hour, roots in tumbler; No. 44, control—under bell jar 1 hour, roots in tumbler. The plants were 22 days old when treated. The first reading was taken in the various cultures after the roots had been in the distilled water subsequent to the treatment for the following periods (but to these periods should be added the time the cultures were under the bell jar, for the roots were in the distilled water during that interval also): No. 39, 2 hours and 12 minutes; No. 40, 2 hours and 23 minutes; No. 41, 2 hours and 20 minutes; No. 42, 2 hours and 30 minutes; No. 43, 2 hours and 11 minutes; No. 44, 2 hours and 23 minutes.

the roots meanwhile remaining practically normal, transpiration no doubt still continued. It remains an open question, however, whether such transpiration caused lower conductivity readings, due to the consequent absorption of electrolytes, than would have been the case had there been no, or only slight, transpiration, as in the cases where the tops were removed. The roots in all the cultures remained turgid and practically

TABLE II
EFFECTS OF ILLUMINATING GAS ON THE EXOSMOSIS FROM THE ROOTS OF
PLANTS UNDER VARIOUS CONDITIONS

Culture no.	Treatment	Interval in dist. H ₂ O before first reading	Conductivity Readings*				Increase‡ over dist. H ₂ O after 88 hrs.
			After first interval	After 24 hrs.	After 88 hrs.		
1 and 2	Controls in dist. H ₂ O, no gas treatment. Tops cut off immediately after placing roots in dist. H ₂ O.....	10 hrs.	33.6	37.9	41.8†	35.8†	
3	Illuminating gas 1 hr.; roots in tumbler. Tops not cut off	1 hr., 17 min.	18.4	38.9	56.7	50.7	
4	Illuminating gas 1 hr.; roots in tumbler. Tops cut off immediately after exposure.....	1 hr., 23 min	23.4	46.6	61.6	55.6	
5	Illuminating gas 1 hr.; roots in tumbler. Tops cut off just before exposure.....	1 hr., 28 min.	18.6	41.2	60.5	54.5	

* Readings represent the values of x on the Wheatstone bridge, resistance in box being 9,110 ohms.

† After 99 hours.

‡ The average reading of the distilled water before roots were placed in it was approximately 6.0.

normal. The higher readings of the treated cultures whose tops had been removed, over those of the untreated controls are to be considered as due to the effect of the illuminating gas, even though in one case only part of the plants was exposed to the agent. The results of this experiment are given in table II.

The results of ether vapor treatment for different periods are seen in fig. 5. An interesting point in this connection is the decline in the curves of the treated plants comparable in

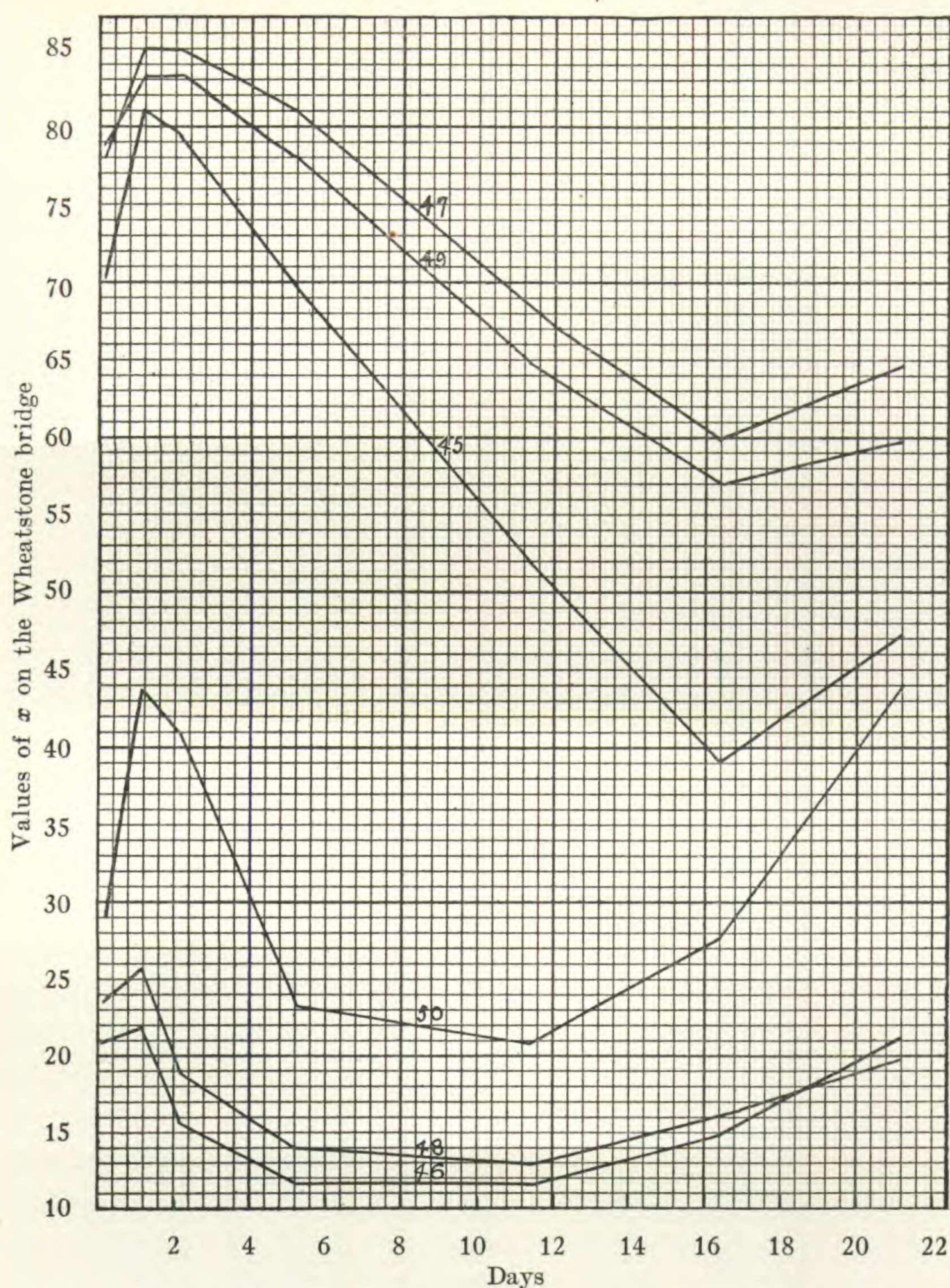


Fig. 5. Conductivity curves of cultures (series 11) in distilled water subsequent to treatment, as follows: No. 45, ether vapor, 30 minutes, roots exposed; No. 46, control—roots exposed under bell jar 30 minutes; No. 47, ether vapor, 1 hour, roots exposed; No. 48, control—roots exposed under bell jar 1 hour; No. 49, ether vapor, 2 hours, roots exposed; No. 50, control—roots exposed under bell jar 2 hours. The plants were 25 days old at the time of treatment. In culture 49, 17cc. of the initial 50cc. of ether remained at the end of the 2 hours. The first reading plotted in each case was taken after the roots had been in the distilled water subsequent to the treatment for the following periods: No. 45, 1 hour and 1 minute; No. 46, 1 hour and 12 minutes; No. 47, 1 hour and 27 minutes; No. 48, 1 hour and 38 minutes; No. 49, 1 hour and 17 minutes; No. 50, 1 hour and 28 minutes.

some respects to that in the curves obtained from normal plants. A distinction should be made here, however, from the causal agency in this decline in conductivity and the anesthetic reversibility that Osterhout ('13) describes. The decline in the curve indicates that the absorption of electrolytes by roots occurs at a greater rate than they are excreted, for both processes, absorption and excretion, are undoubtedly going on and the curve represents the proportionate amounts of each for any given time. Thus if A represents the excretion and B represents the absorption, the curve declines when B is greater than A, and inclines when A is greater than B. Hence the curve may be represented as $A - B = C$, where C represents the number of ions or charge-carriers in the solution. The tops of the treated plants showed no visible effects whatever when compared with the controls. The roots of No. 45 were very slightly affected, but those of Nos. 47 and 49 were considerably so and to about an equal degree, as shown by flaccidity, root coloration, and the colored and turbid appearance of the medium; the tops, however, continued normal for 21 days after the treatment. Hence the metabolic processes no doubt proceeded unimpaired in many respects, as did also transpiration. The decline of the conductivity curve therefore represents merely a partial return to normal conditions. But the higher conductivity of the medium shows greater exosmosis than from the normal plants. This is due to the unalterable and invariable (and not reversible) effect of the anesthetic upon certain cells. Culture 50 shows in the higher position of its curve, as compared with the other controls, an effect that is no doubt due to the 2-hour exposure of the roots to the air in the bell jar.

As seen in fig. 6 no marked results followed the ether application for one-half to two hours when the roots were in the water during the treatment, though a slight rise is evident for the culture exposed 2 hours. No visible effects were produced on either the tops or roots.

Comparing the effects on plants of an ether vapor-saturated atmosphere with those produced by an illuminating gas-saturated atmosphere, it is thus seen that illuminating gas is much

more injurious than is ether vapor under the conditions of the experiment. Equal amounts of each might give different results, however. The gas used was a mixture of water- and coal-gas with a specific gravity of .62 as compared with air;

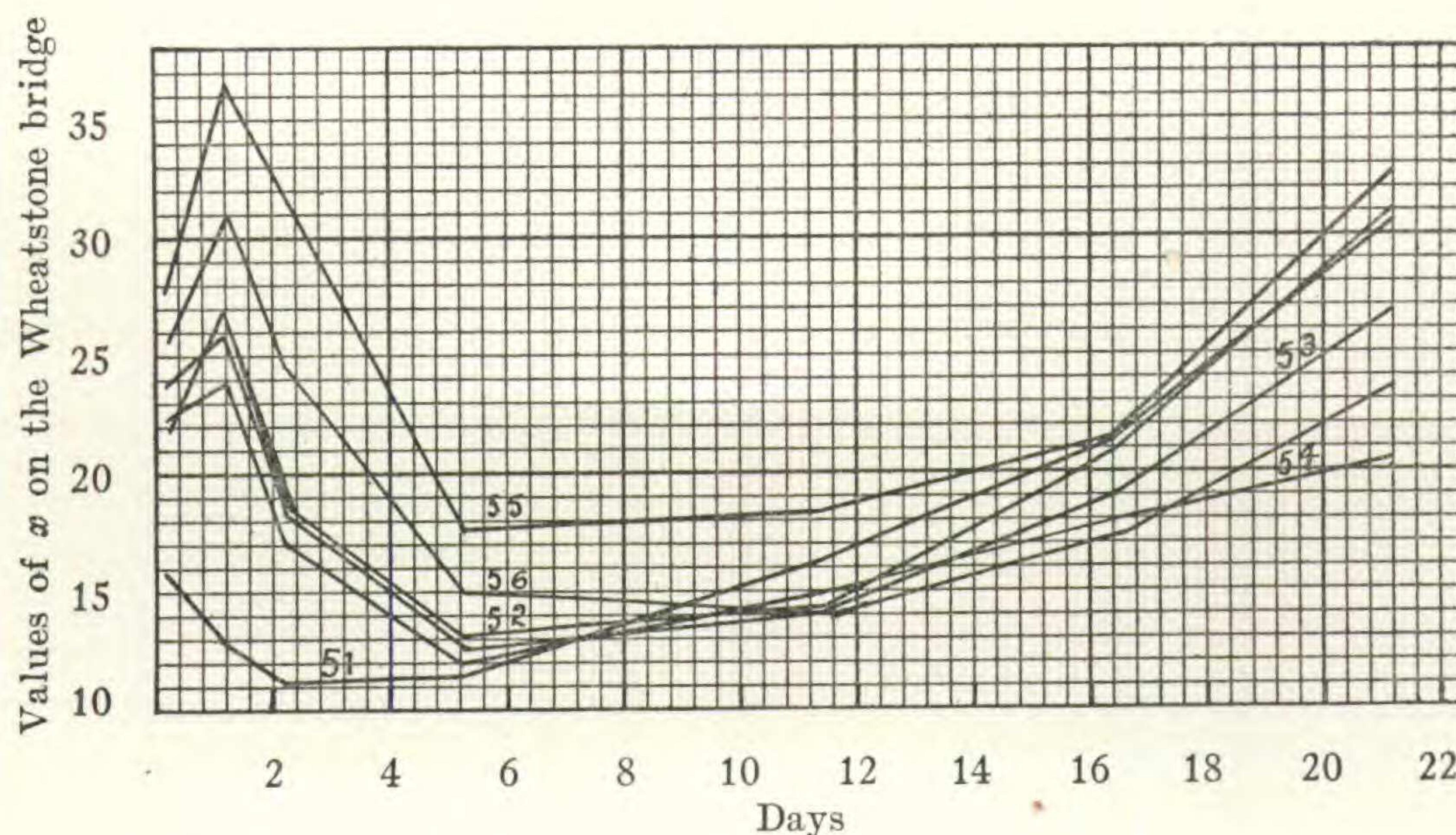


Fig. 6. Conductivity curves of cultures (series 11) in distilled water subsequent to treatment, as follows: No. 51, ether vapor, 30 minutes, roots in tumbler; No. 52, control—under bell jar 30 minutes, roots in tumbler; No. 53, ether vapor, 1 hour, roots in tumbler; No. 54, control—under bell jar 1 hour, roots in tumbler; No. 55, ether vapor, 2 hours, roots in tumbler; No. 56, control—under bell jar 2 hours, roots in tumbler. The plants were 25 days old at the time of treatment. In culture 55, 60cc. of the initial 100cc. of ether remained at the end of the 2 hours. The following periods represent the time elapsing in the various cultures between the removal of the cultures from the bell jar and the taking of the first reading (to which period should be added the duration of treatment, for the roots were in the distilled water during that time also): No. 51, 1 hour and 24 minutes; No. 52, 1 hour and 46 minutes; No. 53, 1 hour and 51 minutes; No. 54, 2 hours and 6 minutes; No. 55, 1 hour and 39 minutes; No. 56, 1 hour and 50 minutes.

one of the daily samples analyzed by the gas company (the officials of which kindly supplied the writer with the data and informed him that they may be considered an approximately fair average) showed the following constituents:

CO ₂	3.0%
O ₂5%
Illuminants (unsaturated hydrocarbons, e. g., ethylene and acetylene)	7.0%
CO	16.1%
CH ₄	25.6%
H ₂	42.8%
N ₂	5.0%

Crocker and Knight ('08), in their work on the question of injury by illuminating gas and its constituents, concluded that "there is much evidence that indicates that the toxic limits of illuminating gas upon these flowers [carnations] is determined by the ethylene it contains." They used a small greenhouse of 1.69 cubic meters' capacity in which they placed potted plants for varying intervals, specified amounts of gas being introduced. The buds were easily injured but the vegetation was apparently not affected even after an exposure of about 72 hours, during which 10 liters of gas had been introduced, 2 or 4 liters at a time. The method was therefore somewhat different from the one employed by the author, in which the plants were placed in an atmosphere saturated with illuminating gas, but for a much shorter period. The underlying cause of the effect in both cases, however, is probably the same.

The etherization of plants as a practical process has been in operation for many decades, especially as a means of hastening the activities of plants, particularly of bringing them into bloom earlier. Some experimental work has also been done, as we have seen, on the effect of such treatment (though in most cases only when the anesthetics were in solution) upon the exosmosis of non-electrolytes, as determined by various methods, from plant or animal cells. It is interesting, therefore, to observe the exosmotic phenomena of electrolytes when the plants are anesthetized under various conditions.

To determine whether the amount of substance excreted corresponded to the conductivity readings, the water in the tumblers was evaporated and the residue weighed. The following are the results:

Total wt. of substance from illuminating gas-treated cultures (Nos. 33, 35, 37, 39, 41, and 43)	0.1514 grams
Total wt. of substance from ether-treated cultures (Nos. 45, 47, 49, 51, 53, and 55)	0.0674 grams
Total wt. of substance from the 12 controls.....	0.1077 grams
Total wt. of substance from 6 controls, therefore.....	0.0538 grams

We may obtain a rough basis for estimating this residue in terms of NaCl by comparing the figures just given with the data on a previous page which gave the corresponding specific conductivity values for some values of x on the Wheat-

stone bridge and also for various concentrations of NaCl. Thus 0.15 gram residue was obtained from 1500 cc. of the media from illuminating gas-treated cultures. This is equivalent to 0.10 gram in 1 liter, which in terms of NaCl would be

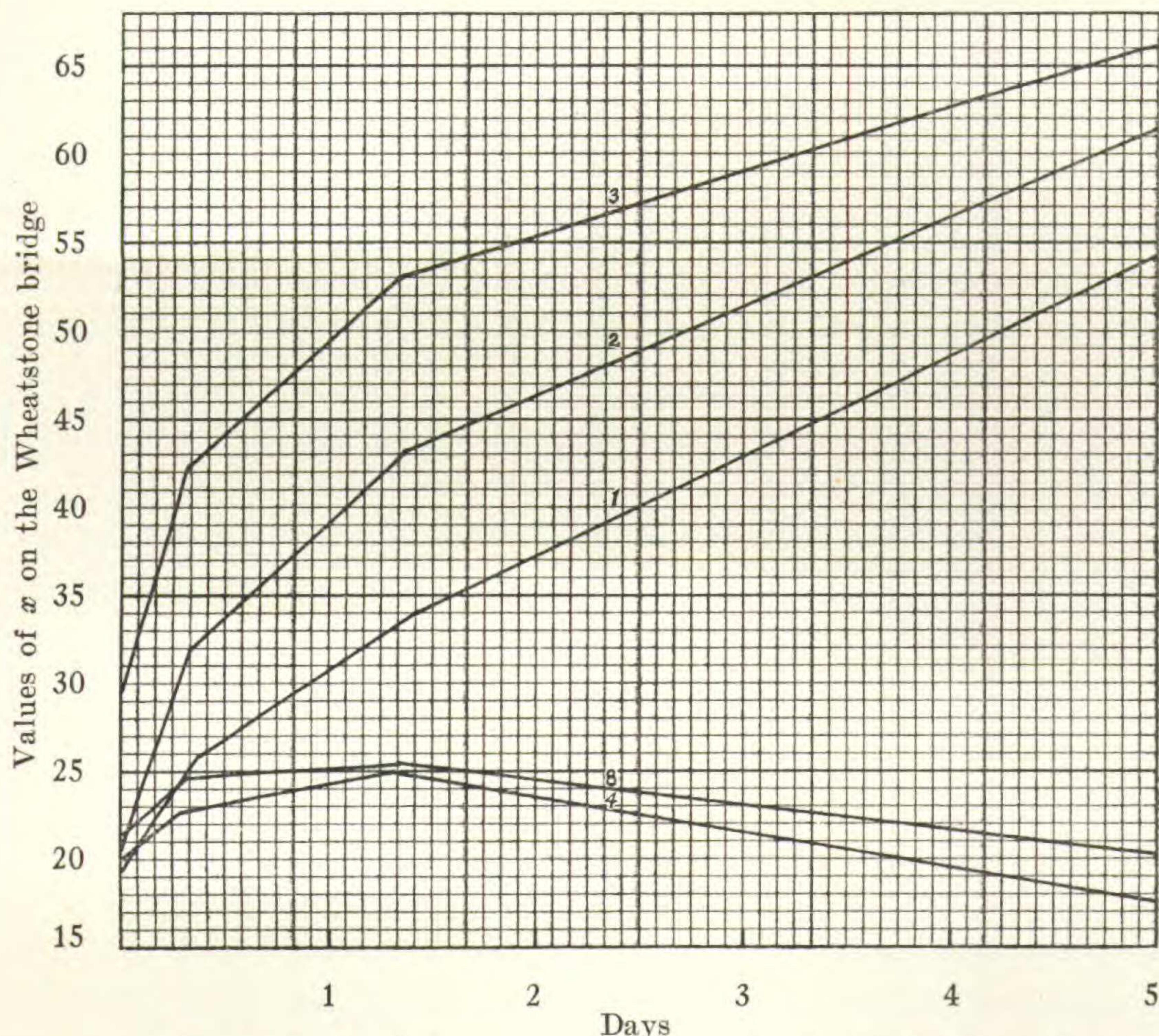


Fig. 7. Conductivity curves of cultures (series 14) in distilled water subsequent to treatment, as follows: No. 1, temperature of -6 to $-2^{\circ}\text{C}.$, 1 hour, roots in tumbler; No. 2, temperature of -6 to $-2^{\circ}\text{C}.$, 2 hours, roots in tumbler; No. 3, temperature of -6 to $-2^{\circ}\text{C}.$, 3 hours, roots in tumbler; No. 4, control—room temperature, roots in tumbler; No. 8, control—room temperature, roots in tumbler. The plants were 23 days old at the time of treatment. The first reading was taken in each case after the roots had been in the distilled water for the following respective periods (cultures 1-3 were being treated during part of that time): No. 1, 1 hour and 47 minutes; No. 2, 2 hours and 29 minutes; No. 3, 3 hours and 45 minutes; No. 4, 3 hours and 6 minutes; No. 8, 2 hours and 2 minutes.

approximately N/500. The specific conductivity of N/500 NaCl is about 25×10^{-5} , the x value of which on the Wheatstone bridge is 85. The average final reading of the 6 cultures treated with illuminating gas is 79.5. Hence the residue in terms of NaCl would be in the neighborhood of N/500.

VI. EFFECTS OF HIGH AND LOW TEMPERATURES

After the preliminary experiments noted above on the effect of heat had been carried out it was desired to study the question further and determine the resulting exosmosis curves at the extreme temperatures, high and low. The preliminary experiments had involved temperatures requiring a considerable time interval to produce positive results. The data now to be presented concern temperatures sufficient in themselves to effect decided injury in a very short period. By varying the time factor, therefore, results could readily be obtained on both sides of the point of injury.

For the experiment, the results of which are plotted in fig. 7, cultures were set out of doors for the time indicated, directly exposed to the winter temperature. The tops showed some signs of freezing after a few moments, but the effects did not become noticeably worse until the cultures were brought inside, when all the plants in each culture immediately drooped over the wire supports and became entirely limp, and soon died. The tops did not yellow, but retained the green color after death. Except for the root tips of the plants in No. 3, which were slightly brown at the end of 5 days, all the roots of the treated plants remained turgid, white, normal, and in healthy condition. This is interesting in view of the fact that while no ice was formed in No. 1, there was a slight fringe of it between the water and the tumbler in No. 2, and a hollow cylinder of ice one-fourth inch thick formed next to the tumbler wall in No. 3. In the last-mentioned culture there was also a film of ice over the surface of the water and the roots were frozen to the ice mass so that on lifting the plants from the tumbler the mass of ice adhered to the roots. The first readings were taken only after the ice had melted. The temperature at first was -6°C . but by the end of the first hour it had risen to -2°C ., where it remained practically constant for the balance of the interval.

At a temperature of -6.5°C . it is seen by reference to fig. 8 that while for exposures of the plants alone (the roots being out of the water) of 2 and 3 minutes, marked exosmosis imme-

diately results, exposures of 1 minute or $\frac{1}{2}$ minute produce no results. Culture 13 has rather high exosmosis for a control,

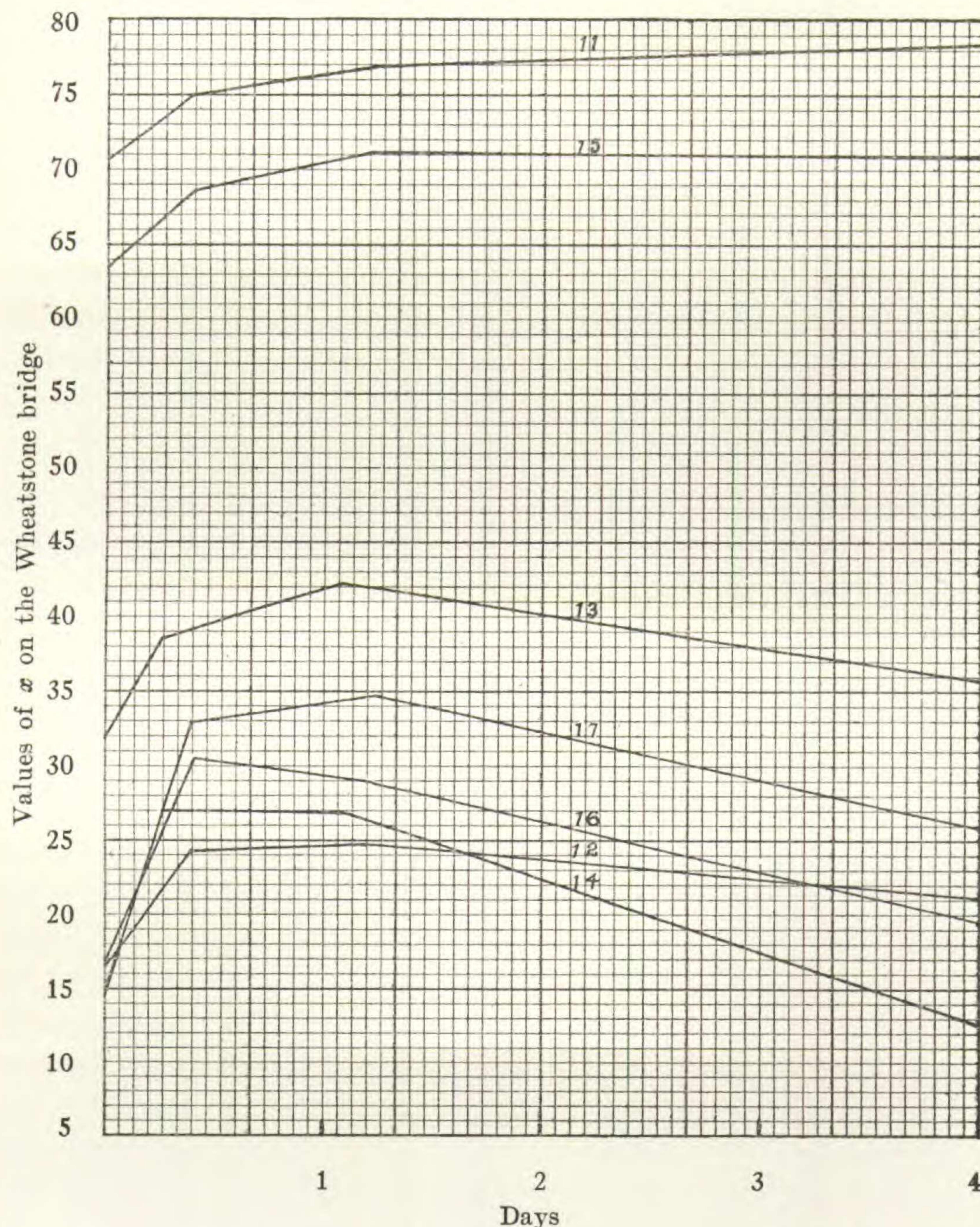


Fig. 8. Conductivity curves of cultures (series 14) in distilled water subsequent to treatment, as follows: No. 11, temperature of $-6.5^{\circ}\text{C}.$, 3 minutes, roots exposed; No. 12, control—roots exposed to laboratory temperature 15 minutes; No. 13, control—roots exposed to laboratory temperature 30 minutes; No. 14, control—roots exposed to laboratory temperature 5 minutes; No. 15, temperature of $-6.5^{\circ}\text{C}.$, 2 minutes, roots exposed; No. 16, temperature of $-6.5^{\circ}\text{C}.$, 1 minute, roots exposed; No. 17, temperature of $-6.5^{\circ}\text{C}.$, one-half minute, roots exposed. The plants were 24 days old at the time of treatment. The first reading was taken in each case after the roots had been in the distilled water for exactly 30 minutes subsequent to the treatment.

but this is readily accounted for by the exposure of its roots to the atmosphere of the laboratory for 30 minutes, a condition noted in other cases above.

Cultures 9 and 10 of this series, the results from which are not represented because both tops and roots were killed outright, the resulting exosmosis therefore being immediate and high (x being about 88.0 cm.), were exposed for 15 and 33 minutes respectively to a temperature of -6.5°C ., the roots being out of the medium. In a very short time, on returning them to the laboratory, the tops wilted and drooped over the supporting wires and the roots became very flaccid. In the case of No. 11, however, an interesting gradation or intermediate condition was observed between it and Nos. 9 and 10 on one hand and between it and the controls on the other. While the tops in No. 11 wilted and drooped somewhat soon after being returned to the higher temperature of the laboratory, they did not become entirely limp and the roots were only slightly less turgid than those of the controls. Even after 4 days the tops of No. 11 were not drooping much, though the tips of the branches and the upper leaves were dead; the lower part of the stems and the lower leaves remained green and normal. The lateral roots and the older part of the main roots remained nearly normal, but the tips of the latter were flaccid and shrunken for about 2 inches. Culture 15 showed a very slight flaccidity in the tops and roots soon after the treatment, and after 4 days some of the younger leaves and the tips of the older leaves were blackened, curled, and dried somewhat, but the great part of the tops remained normal in appearance; the roots were slightly flaccid at the tips, but were in general practically normal. Cultures 12, 13, 14, 16 and 17 were normal in respect to both roots and tops.

The interval between 15 and 30 minutes is shown in fig. 9 to be the critical period for the pea plants exposed in a tumbler to a temperature of from -2°C . to -2.5°C ., for an exposure of 30 minutes caused considerable exosmosis, while one of 15 minutes gave a curve approximately that for normal plants.

To contrast the effects of low and high temperatures, Nos.

25-28 inclusive are plotted in the same figure with Nos. 18 and 23. With plants enveloped in a steam bath the injury, as expected, is very speedy and effective. Even one-half minute when the roots are exposed—causes immediate and marked

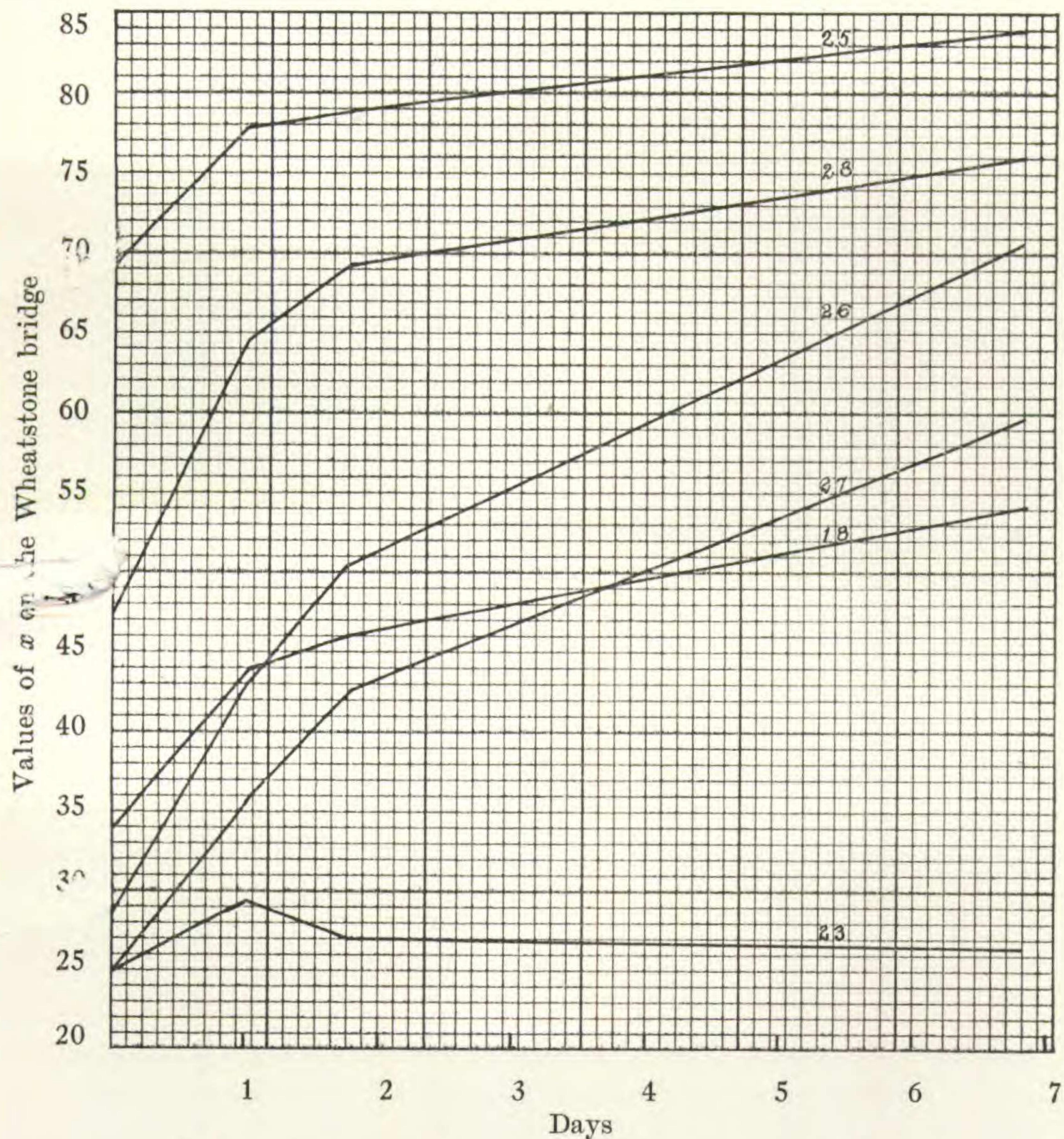


Fig. 9. Conductivity curves of cultures (series 14) in distilled water subsequent to treatment, as follows: No. 18, temperature of $-2.5^{\circ}\text{C}.$, 30 minutes, roots in tumbler; No. 23, temperature of $-2.0^{\circ}\text{C}.$, 15 minutes, roots in tumbler; No. 25, steam, one-half minute, roots exposed; No. 26, steam, 2 minutes, roots in tumbler; No. 27, steam, 1 minute, roots in tumbler; No. 28, steam, 10 minutes, roots in tumbler. The plants were 29 days old at the time of treatment. The first reading was taken in each case after the roots had been in the distilled water for the following respective periods (the cultures whose roots were in tumblers during the treatment were likewise in the distilled water): No. 18, 4 hours and 49 minutes; No. 23, 4 hours and 41 minutes; No. 25, 4 hours and 43 minutes; No. 26, 4 hours and 49 minutes; No. 27, 4 hours and 31 minutes; No. 28, 4 hours and 12 minutes.

exosmosis which is greater than that caused by a 10-minute exposure when the roots are in distilled water meanwhile. The condition of the plants immediately after the treatment and again after 7 days is given in table III. Here again is illus-

TABLE III
CONDITION OF PLANTS AFTER EXPOSURE TO VARIOUS TEMPERATURES

Culture no.	Condition of tops	Condition of roots
Condition of plants immediately after the treatment:		
18	Considerably flaccid and drooping.....	Entirely normal
23	Very slightly drooping, nearly normal.....	Entirely normal
25	Drooping considerably.....	Normal
26 and 27	Drooping, green and damp.....	Normal
28	Drooping, green and damp.....	Apparently practically normal
Condition of plants 7 days after the treatment:		
18	About half dead and half alive; 3 live stems with green, normal leaves.....	Entirely normal
23	Almost normal; tips of a few stems killed and some slightly injured, but some stems normal throughout; a few blackened leaves, but for the most part stems and leaves green and normal.....	
25	Dead.....	Entirely normal
26 and 27	Dead.....	Only very slightly flaccid and nearly normal in appearance
28	Dead.....	Practically normal in appearance
		Almost normal

trated, therefore, the case where there is considerable exosmosis without very marked visible effects resulting to the root tissues.

The effects of moist heat, as graphically represented in fig. 9, having been considered, we may now turn our attention to fig. 10, where the results are plotted of an exposure of plants to dry heat for short intervals, both with the roots directly exposed and with the roots remaining in the tumbler of water during the treatment.

It is seen that definite and positive exosmosis is obtained after a 4-minute exposure of the unprotected roots. The decline of the curve of No. 29, roots exposed for 2 minutes, is probably best accounted for by assuming greater hardihood of the plants in that culture, or that some condition effected an increase in transpiration. A 1-minute exposure (No. 30) pro-

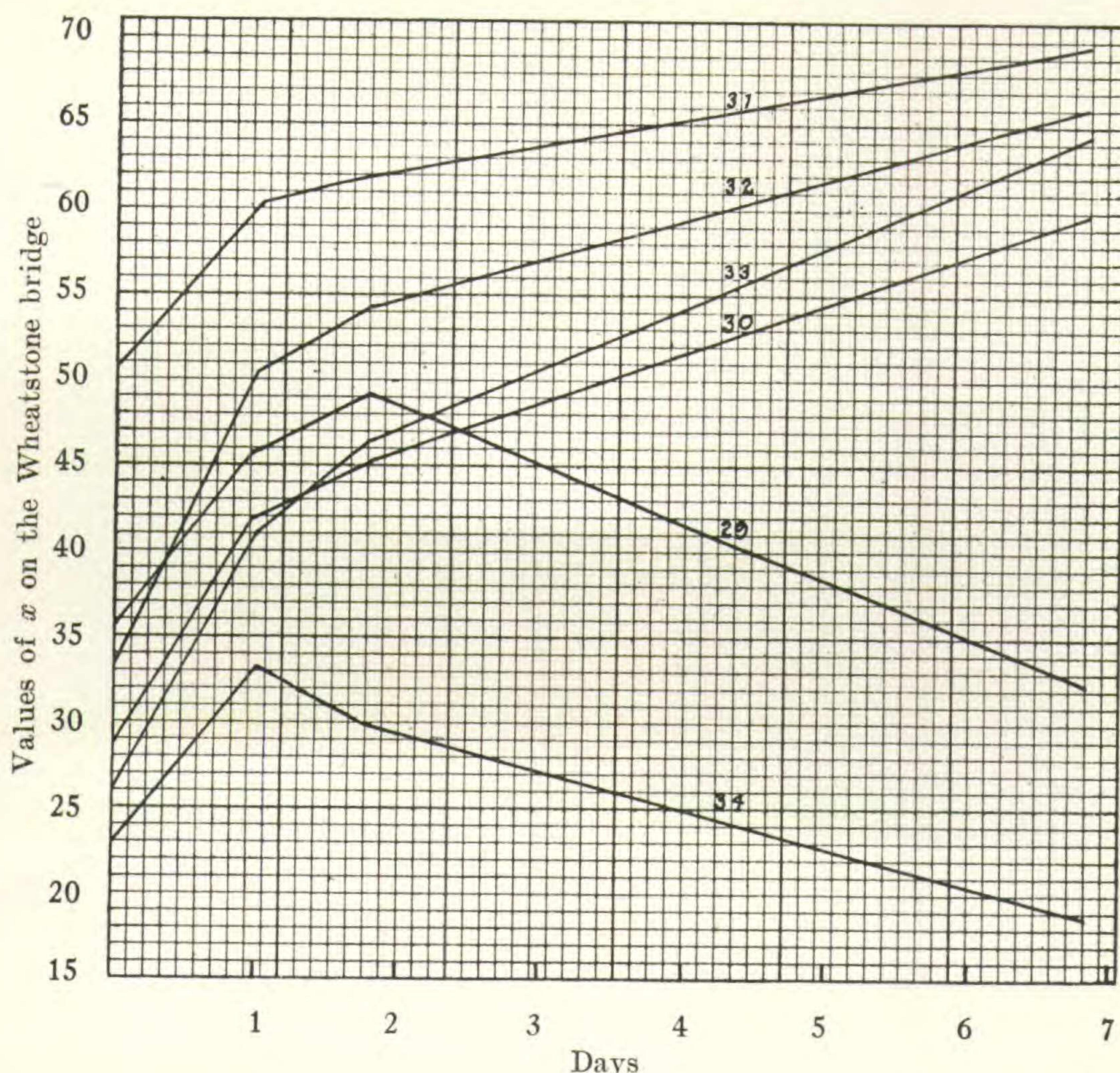


Fig. 10. Conductivity curves of cultures (series 14) in distilled water subsequent to treatment, as follows: No. 29, temperature of 92°C., 2 minutes, roots exposed; No. 30, temperature of 92°C., 1 minute, roots exposed; No. 31, temperature of 92°C., 4 minutes, roots exposed; No. 32, temperature of 92°C., 2 minutes, roots in tumbler; No. 33, temperature of 92°C., 4 minutes, roots in tumbler; No. 34, control—roots in tumbler at laboratory temperature. The plants were 29 days old at the time of treatment. The first reading was taken in each case after the roots had been in the distilled water for the following respective periods (the cultures whose roots were in tumblers during the treatment were likewise in the distilled water during that time): No. 29, 2 hours and 47 minutes; No. 30, 2 hours and 48 minutes; No. 31, 2 hours and 44 minutes; No. 32, 2 hours and 45 minutes; No. 33, 2 hours and 46 minutes; No. 34, 2 hours and 46 minutes.

duced less exosmosis at the beginning than was the case in No. 29, but finally caused more. The same irregularity is also noticed in Nos. 32 and 33. These irregularities near the boundary line of endurance have been discussed above. The tops of Nos. 29-33 were killed by the treatment, but the roots of all remained practically normal in appearance except in No.

31, where the tips were slightly shrunken at first but became almost normal in the water after 7 days.

In the temperature experiments we have thus used the extremes of temperature and have reduced the interval of exposure in order to approach the point at which the effect is just evident.

VII. EFFECTS OF ANESTHETICS IN SOLUTION

Having seen some of the effects of anesthetic vapors, we may turn our attention next to the results obtained with anesthetics in solution. In the investigations of others pertaining to the effect of anesthetics, already cited, the result has been almost universally noted that small amounts of anesthetics decrease the exosmosis of coloring matters, etc., while toxic amounts increase it. In most cases this exosmosis was explained on the basis of an alteration in the plasma membrane, small amounts of the anesthetics presumably reducing the permeability and large amounts increasing it. But a point worthy of note is that wherever such effects have been determined the substance under observation was either a colored compound or one of complex organic nature.

Thus Czapek ('11) used the myelin-formation of a tannoid substance, anthocyan, as a basis of observation. From the standpoint of a physical phenomenon, i. e., the lowering of surface tension, his experiments beautifully illustrated the principle under consideration. But from the standpoint of exosmosis in the broader sense we must include electrolytes (salts, bases, and acids) as well as tannin compounds in any discussion dealing with agents affecting exosmosis, and while the critical concentrations which he determined are undoubtedly characteristic of the plants and the compounds studied, the results given herewith show that they are not the limiting concentrations which effect the exosmosis of electrolytes from the roots of certain plants. The limiting concentrations which he found are given in table iv.

Czapek believed the permeability of the plasma membrane was altered under the influence of alcohols, ethers, etc., so that abnormal exosmosis occurred. Whatever may be the expla-

TABLE IV
CRITICAL CONCENTRATIONS (THOSE JUST SUFFICIENT TO CAUSE EXOSMOSIS)
OF SOME ORGANIC COMPOUNDS AS DETERMINED BY
CZAPEK FOR CERTAIN PLANTS

Agent	Plant	Concentration of agent	Surface tension*
Methyl alcohol	<i>Echeveria</i>	18% aqueous solution (by volume)	.71
Ethyl alcohol	<i>Echeveria</i> and <i>Saxifraga</i>	10-13% aqueous solution (by volume).....	.65-.70
Ethyl ether	<i>Echeveria</i> and <i>Viola</i>	$\frac{1}{4}$ - $\frac{1}{2}$ saturated aqueous solution acting for 24 hrs.....	.61-.71
Chloroform	<i>Echeveria</i>	Saturated aqueous solution†.....	.98
Chloral hydrate	3.09% aqueous solution.....	.93
Ethyl acetate	Saxifraga hairs and variegated leaves of <i>Op- tismenus imbe- cillus</i>	3% aqueous solution acting for 12 hrs.....	.69-.73
Ethyl acetate	Red beets.....	2% aqueous solution acting for 12 hrs.....	

* In terms of water as unity. † After 24 hours the cells had lost all tannin.

nation for the phenomena observed, it will be seen by comparing the results in the following experiments with the data just given that the limiting values found for the exosmosis of electrolytes do not at all correspond to the values found by Czapek for the exosmosis of the tannoid substance.

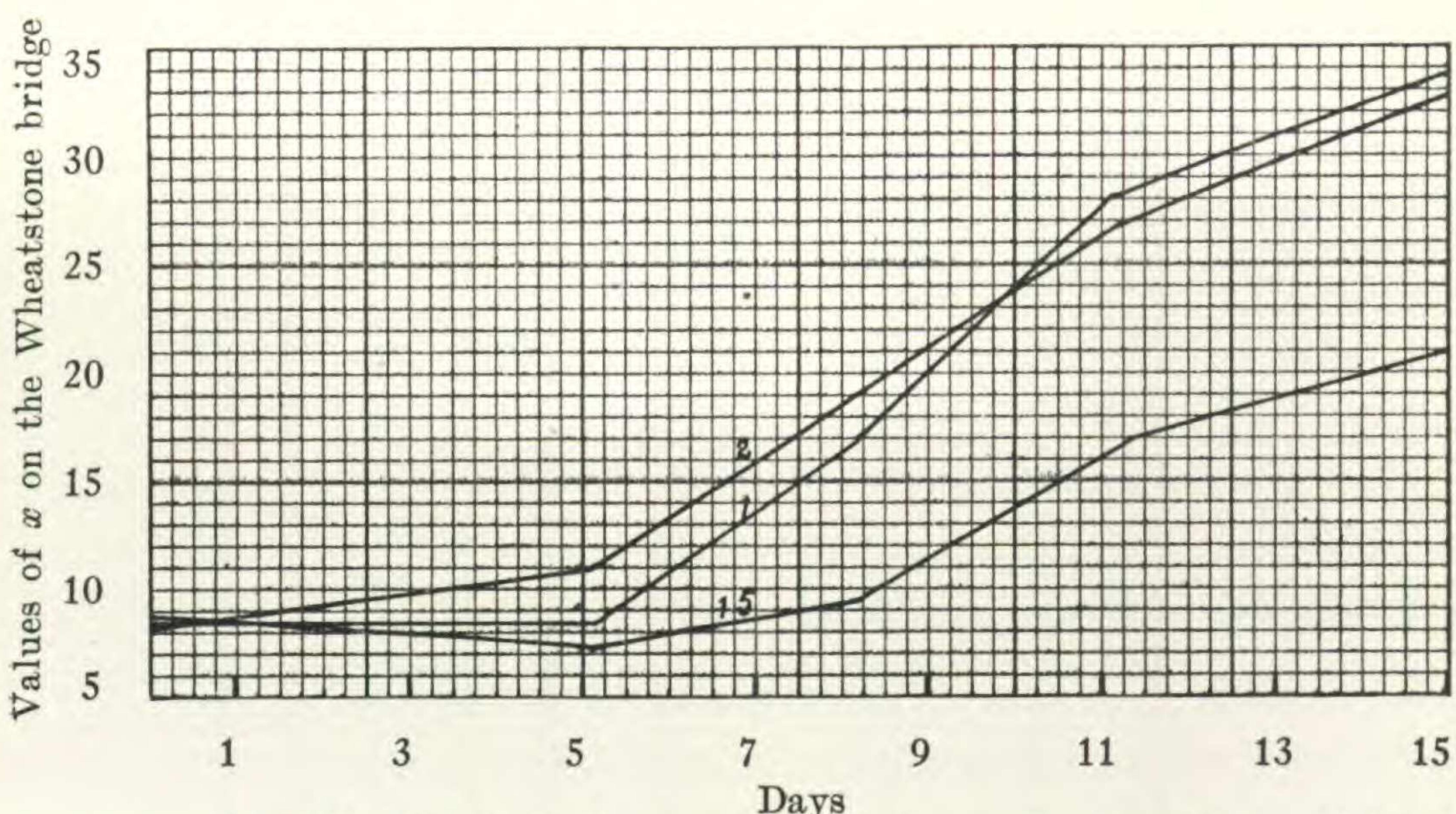


Fig. 11. Conductivity curves of cultures (series 15) in distilled water subsequent to treatment, as follows: No. 1, ether vapor, 15 minutes, roots exposed; No. 2, 1 per cent ether in water, 15 minutes; No. 15, control—roots exposed under bell jar 15 minutes. The plants were 31 days old when treated. In culture 1, 11cc. of the initial 15cc. of ether remained after the 15-minute exposure. In Nos. 1 and 2, the first reading was taken exactly 30 minutes, and in No. 15, 48 minutes, after the roots were placed in the distilled water.

In fig. 11 are shown the results with ether in distilled water and, for comparison, also with ether vapor for the same period. The curves for the ether-treated cultures are closely parallel for the entire period of observation of 15 days, and both are

TABLE V
EFFECTS OF VARIOUS ANESTHETICS ON THE EXOSMOSIS FROM
THE ROOTS OF PLANTS
(See curves in fig. 12)

Vapor Treatment, Roots Exposed			
Anesthetic	Time of exposure	Culture no.	Resulting exosmosis
Ether	30 minutes	3	{ About the same in Nos. 3 and 5
Illuminating gas	15 minutes	5	
Illuminating gas	30 minutes	7	
Chloroform	30 minutes	9	
Treatment with Anesthetics Dissolved in Water			
Ether, 4%	30 minutes	4	High
Ether, 4%	Throughout experiment	11	Highest
Ether, 10%	30 minutes	12	Higher
Illuminating gas-saturated sol'n.	15 minutes	6	Medium low
Illuminating gas-saturated sol'n.	30 minutes	8	Like control
Illuminating gas-saturated sol'n.	Throughout experiment	13	Medium low
Illuminating gas-saturated sol'n. frequently re-saturated	30 minutes	14	Slightly above control
Chloroform, 4%	30 minutes	10	Very high
Controls			
Roots exposed to the air under a bell jar 30 minutes		16	High for control
Roots exposed to the air under a bell jar 30 minutes		17	High for control
Roots not exposed, but in water from first		18	Normal for control

above that of the control. The excretion was nil during the first half hour and at the end of 5 days it was scarcely more, though it may have risen and fallen in the meantime, as no readings were taken in the interim. After 5 days a greater rise in the conductivity curve occurred with the ether-treated cultures than with the control; no apparent effects, however, were produced on either the tops or roots.

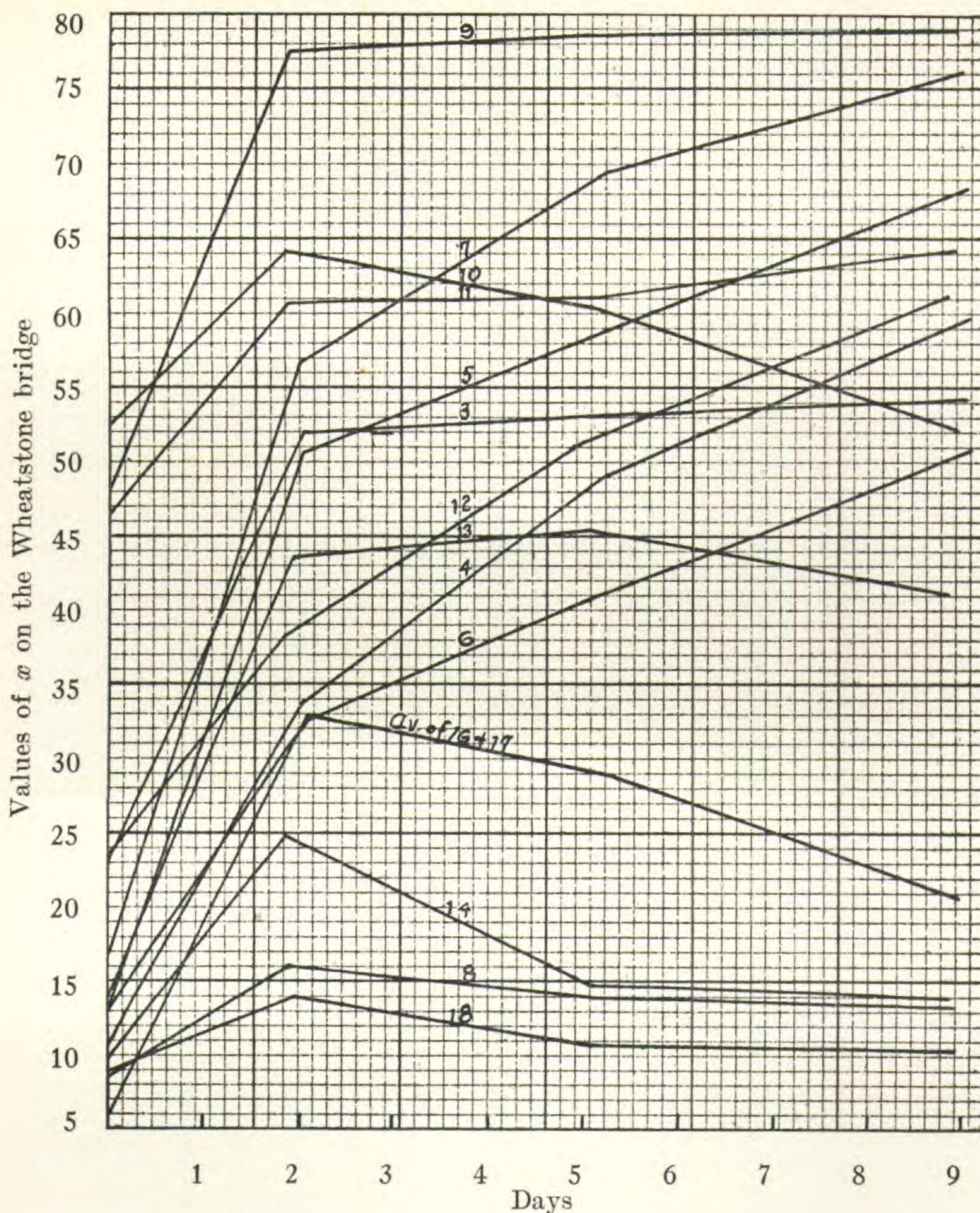


Fig. 12. Conductivity curves of cultures (series 15) in distilled water subsequent to treatment, as follows: No. 3, ether vapor, 30 minutes, roots exposed; No. 4, 4 per cent ether in water, 30 minutes; No. 5, illuminating gas, 15 minutes, roots exposed; No. 6, distilled water saturated with illuminating gas, 15 minutes; No. 7, illuminating gas, 30 minutes, roots exposed; No. 8, distilled water saturated with illuminating gas, 30 minutes; No. 9, chloroform vapor, 30 minutes, roots exposed; No. 10, 4 per cent chloroform in water, 30 minutes; No. 11, 4 per cent ether in water, to the end of the experiment; No. 12, 10 per cent ether in water, 30 minutes; No. 13, distilled water saturated with illuminating gas, to end of experiment; No. 14, distilled water saturated with illuminating gas, 30 minutes (frequently saturated); No. 16, control—roots exposed 30 minutes under bell jar; No. 17, control—roots exposed 30 minutes under bell jar; No. 18, control—roots placed directly into distilled water. The percentages given above refer to volume-per cent. The plants were 37 days old when treated. The first reading was made after the roots had been in distilled water 30 minutes (No. 10, 36 minutes). In all cases treatment preceded placing of the roots in distilled water (conductivity of which was determined except in the cases of Nos. 11, 13, and 18). In culture 3, 17cc. of the initial 25cc. of ether remained at the end of the 30-minute exposure; in culture 9, 23.5cc. of the initial 25cc. of chloroform remained.

To show the comparative effects on the exosmosis from the roots of plants treated with ether, chloroform, and illuminating gas—both when applied as vapor and when introduced into the water—the conductivity curves of fig. 12 were plotted. The results, somewhat classified, are also given in table v. It will be seen that the quantity of anesthetics used and the duration of treatment varied in individual cases.

The indications are, therefore, that for an equal exposure the vapors range in order of effectiveness as follows: ether, least; illuminating gas, more; and chloroform, most. The difference in effectiveness between the ether and the chloroform is especially interesting, more so when we note that 8 cc. of ether were used and only $1\frac{1}{2}$ cc. of chloroform. This would seem to be in harmony with the findings of Graham ('14); he was able to produce liver necrosis by some aliphatic halogen substituted compounds, but not by ether or chloral hydrate.

As regards the fact that No. 11 (4 per cent ether, remaining in the water) has a higher curve than No. 12 (10 per cent ether for 30 minutes) and especially at the beginning, it should be stated that in the case of Nos. 4, 6, 8, 10, 12, and 14, the treatment was given while the roots were in distilled water plus the anesthetics. Following the treatment the roots, after rinsing, were placed in distilled water, and at the end of one-half hour the first reading was taken. In the case of Nos. 11 and 13 the water containing the anesthetic was not replaced by fresh water and the first reading was taken one-half hour after the treatment began. Since the exosmosis during the first half hour is unusually rapid as a result of anesthetic treatment, it will be seen that in replacing the medium at the end of that period, the excreted material was thus discarded for that interval. Hence, such curves represent a secondary exosmosis. The curve of No. 11, therefore, is for total exosmosis, while that of No. 12 is for partial exosmosis.

The condition of the cultures which furnished the results plotted in fig. 11 is given for various periods in table vi.

In fig. 13 the secondary exosmosis after the first half hour is graphically represented for some organic compounds in considerable concentration. The purpose was, of course, to use a

concentration sufficiently effective to give results in a short interval of time. After the treatment the roots were rinsed and placed in distilled water. It is interesting to note that the alcohols used were only slowly effective at first, but that

TABLE VI
CONDITION OF PLANTS AFTER TREATMENT WITH ANESTHETICS

Culture no.	Condition of tops		Condition of roots
	Condition of plants 2 days after treatment:		
3 and 4	Slightly subnormal, but almost normal		Somewhat flaccid
5	Practically same as in Nos. 3 and 4 . . .		Practically normal
6	Practically same as in Nos. 3 and 4 . . .		Practically normal, but somewhat flaccid
7	Practically all dead		Somewhat flaccid
8	Almost normal		Slightly flaccid
9, 10, 11, and 12	Normal		Considerably flaccid
13	Normal		Practically normal
14	Normal		Slightly flaccid
16, 17, and 18	Normal		Normal
Condition of plants 9 days after treatment:			
3	Much dried and yellowed		Practically normal
4	Slightly worse than in No. 3		Considerably flaccid
5	Same as in No. 4		Less flaccid than in No. 4
6	Mostly dried up		Practically normal
7	All dried up		Slightly flaccid
8	Practically normal		Practically normal
9	Practically normal		Considerably flaccid
10	Practically normal		Somewhat flaccid
11 and 12	Almost normal		Somewhat flaccid
13 and 14	Normal		Practically normal
16	Slightly subnormal		Practically normal
17 and 18	Normal		Normal

after 8 days the conductivity readings for those cultures were as high as those of the other cultures. Benzol and toluene produced almost identical effects. The effect produced by chloral hydrate remained constant after 1 day. Ethyl acetate and benzaldehyde were especially effective. The condition of the plants at the end of 8 days is given in table VII.

In fig. 14 are shown the effects of smaller amounts of the same substances, the curves of which are exhibited in fig. 13. Here, however, concentrations only one-fourth as great as those previously employed were used, but the chemicals were allowed to remain in the water during the entire period (or until evaporated, as may have been the case with some).

While the alcohols gave a greater effect than the control, they gave no greater exosmosis than one of the controls in the

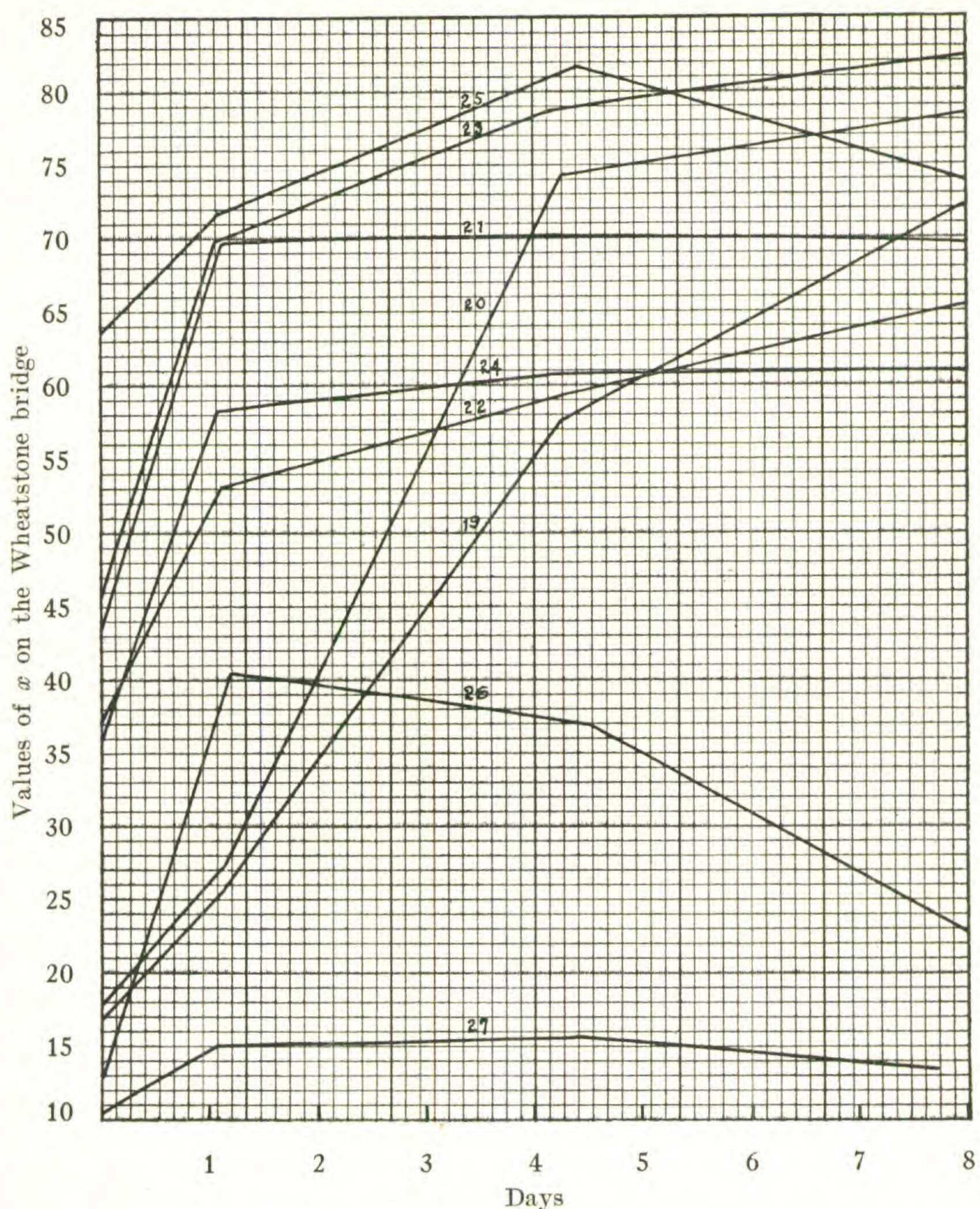


Fig. 13. Conductivity curves of cultures (series 15) in distilled water subsequent to treatment, as follows: No. 19, 4 per cent ethyl alcohol in water, 30 minutes; No. 20, 4 per cent methyl alcohol in water, 30 minutes; No. 21, 4 per cent chloral hydrate in water, 30 minutes; No. 22, 4 per cent benzol in water, 30 minutes; No. 23, 4 per cent ethyl acetate in water, 30 minutes; No. 24, 4 per cent toluene in water, 30 minutes; No. 25, 4 per cent benzaldehyde in water, 30 minutes; No. 26, control—placed directly into distilled water; No. 27, control—placed directly into distilled water. The plants were 38 days old at the time of treatment. The first reading was taken in all cases after the roots had been in distilled water exactly 30 minutes. In the case of the treated plants the roots were in distilled water containing the anesthetic for the specified time, after which they were transferred to the distilled water, the conductivity of which was subsequently determined.

previous figure. The other substances, however, even in the small concentration employed, produced a marked rise in the conductivity of the medium during the first day, after which it remained practically constant. The benzaldehyde and the

TABLE VII
CONDITION OF PLANTS EIGHT DAYS SUBSEQUENT TO TREATMENT WITH
EFFECTIVE CONCENTRATIONS OF ANESTHETICS FOR A SHORT PERIOD

Culture no.	Condition of tops	Condition of roots
19	Normal and in good condition.....	Considerably flaccid
20	Practically normal.....	More flaccid than those of No. 19
21	Somewhat subnormal.....	Somewhat flaccid
22	Some stems considerably affected, others almost normal.....	Considerably flaccid
23	Practically all dead.....	Very flaccid
24	About the same as in No. 22.....	Considerably flaccid
25	Considerably subnormal.....	Very flaccid
26	Normal; many green, vigorous, turgid leaves.....	Practically normal
27	Practically normal.....	Normal

ethyl acetate, which themselves give a high conductivity in aqueous solution, should be considered apart from the other substances, which give no such increase. The two substances mentioned are given here merely for the purpose of comparison with the others employed. A 1 per cent solution of ethyl acetate had a conductivity of 65.2 on the Wheatstone bridge, while that of a similar solution of benzaldehyde was 88.6. These corrections should therefore be applied to the curve values in order to obtain the true value of the exosmosis from the roots in those cultures. The condition of the plants after 8 days is given in table 8.

VIII. EFFECTS OF SUBSTANCES USED SINGLY AND COMBINED IN PAIRS

It is not the writer's purpose here to go into the historical aspect of the increasingly voluminous work on toxic agents, antagonistic action, and balanced solutions, and the numerous related subjects. But since those subjects have assumed such great importance in the realm of physiology it was thought desirable to consider the effect of certain toxic and unbalanced

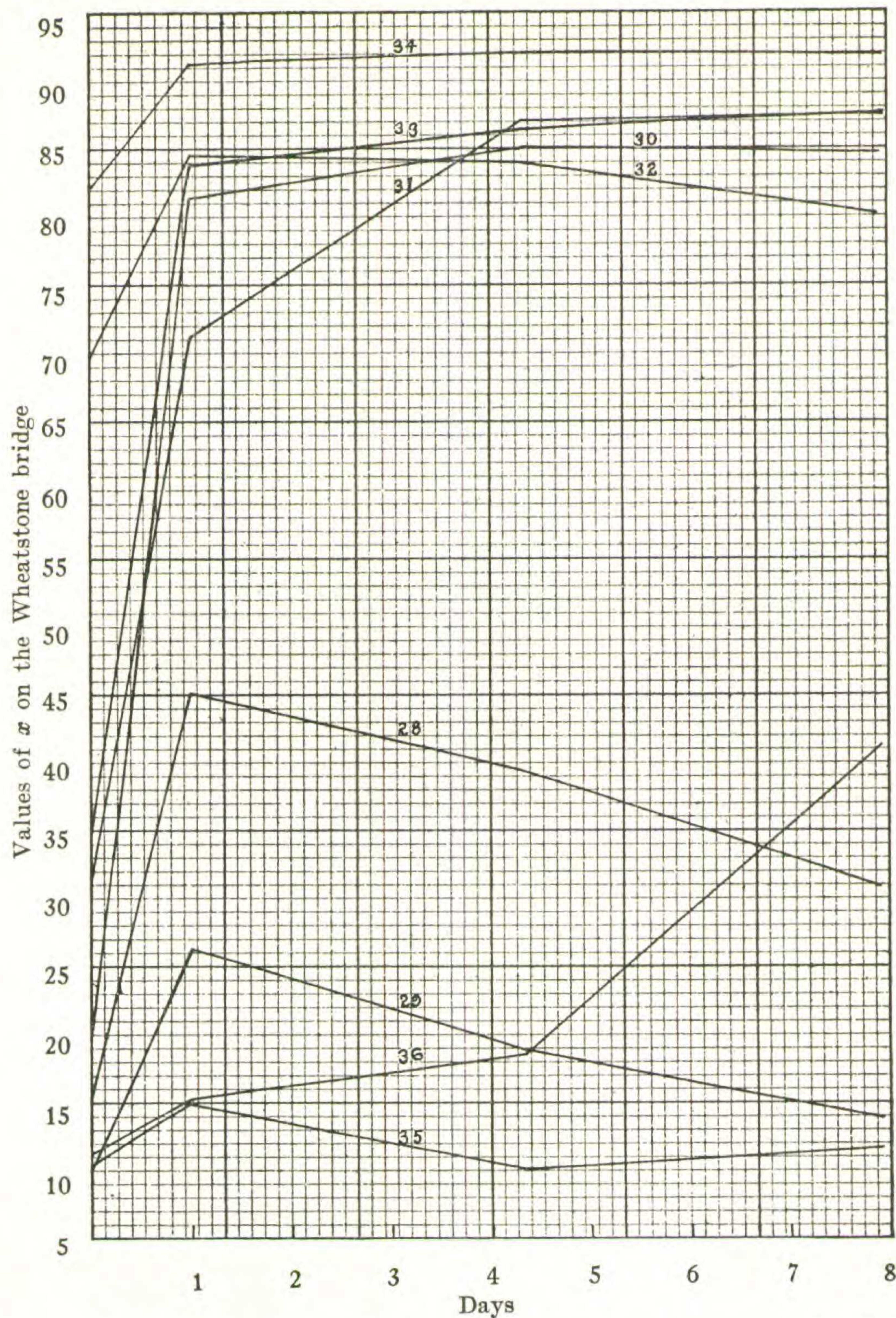


Fig. 14. Conductivity curves of cultures (series 15) in distilled water subsequent to treatment, as follows: No. 28, 1 per cent ethyl alcohol in water, to end of experiment; No. 29, 1 per cent methyl alcohol in water, to end of experiment; No. 30, 1 per cent chloral hydrate in water, to end of experiment; No. 31, 1 per cent benzol in water, to end of experiment; No. 32, 1 per cent ethyl acetate in water, to end of experiment; No. 33, 1 per cent toluene in water, to end of experiment; No. 34, 1 per cent benzaldehyde in water, to end of experiment; No. 35, control—placed directly into distilled water; No. 36, 1 per cent methyl alcohol in water, to end of experiment. The plants were 38 days old at the time of treatment. The first reading was taken in all cases after the roots had been in the distilled water containing the anesthetic exactly 30 minutes. The control, however, was exposed to the distilled water only, the first reading being taken after 30 minutes.

solutions on the exosmosis from plant roots in order to obtain a basis of comparison with the other agents used.

In this connection it might be well to consider more in detail the work of Lillie already referred to in the historical

TABLE VIII
CONDITION OF PLANTS EIGHT DAYS SUBSEQUENT TO TREATMENT WITH LOW CONCENTRATIONS OF ANESTHETICS FOR THE ENTIRE PERIOD

Culture no.	Condition of tops	Condition of roots
28	Somewhat subnormal.....	Slightly flaccid
29	Almost normal.....	Considerably flaccid; tips less flaccid than in No. 28 but upper part more so
30	Dead.....	Considerably flaccid
31	Almost dead.....	Very flaccid
32-34	Practically dead.....	Very flaccid
35	Practically normal.....	Practically normal
36	Practically normal.....	Somewhat flaccid

review. His work on *Arenicola* and the eggs of *Arbacia* pertains largely to the exosmosis of the pigment and the manner in which they were affected by isotonic salt solutions alone and in the presence of various anesthetics. From the effect observed, he concluded that the salts have a permeability-increasing effect on the plasma membrane which is counteracted by the anesthetics. But in dealing with the question of permeability it would seem that we must take into consideration the effect on the exosmosis, not only of any contained pigment, but of electrolytes as well.

It would have been exceedingly interesting, and would have furnished a means of strengthening or shattering his hypothesis, as the case might be, had Lillie also measured the electrical conductivity of the medium in which the *Arenicola* larvae and the *Arbacia* eggs were placed and thus determined whether the electrolytes contained in these organisms behaved as did the pigment. It would seem that the work of Loeb ('03), Peters ('04), and others might be considered as suggesting possibilities for electrolytic determinations along this line with marine organisms. Without such facts at hand any general conclusions in regard to permeability effects based on the coloring matter only must be considered imperfect. What

the reaction may be between the anesthetics and the larval pigment is another question which Lillie does not touch upon. In a recent article Miss Wheldale ('14), in discussing the natural and artificial extracts of plants, states that whereas artificial anthocyanin is soluble in ether the natural anthocyanins are not. May we not have a similar effect in the pigments concerned? Small amounts of the anesthetics may render those pigments insoluble and in that manner prevent their exosmosis rather than by bringing about any considerable alteration of the membrane; larger amounts of the anesthetics would act chemically on the membrane to a point of disintegration sufficient for the physical escape of the pigment.

It will be seen from the following experiments that in the case of roots of *Pisum sativum* certain salts caused a marked exosmosis of electrolytes. In the presence of anesthetics this exosmosis was not decreased or prevented, as Lillie found in the case of the pigments referred to, but was even increased. Hence these results do not indicate any permeability-decreasing action on the part of the anesthetics and are therefore in harmony with the findings of Dixon and Atkins ('13) and others. Another interesting condition is seen in the exosmosis resulting from single and combined salts acting for different periods of time. It was expected that such results would correspond with those obtained on plant-growth studies of antagonistic action between various nutrient and non-nutrient salts. That equally as high, or in some cases higher, exosmosis values were obtained from combined salts as from single salts is an unexpected and interesting result.

As previously indicated, the method used was to place the plants in the various solutions for the period specified and then transfer them, after careful rinsing of the roots, to distilled water in which the conductivity readings were to be taken. It was ascertained that the rinsing was effective in removing electrolytes from the roots. Figures 15 and 16 show the results for the briefer treatments with certain salts, and it is there seen that for a period of treatment less than 17 hours the N/20 $MgCl_2$ has no effect. While in the case of the culture treated for one-half hour with the $MgCl_2$, the conductivity

reading was higher at the end of four days than in the other cultures (2-5) of that group, and continued higher throughout, this fact loses its significance, as far as comparative effects are concerned, when the curve resulting from a 4-hour

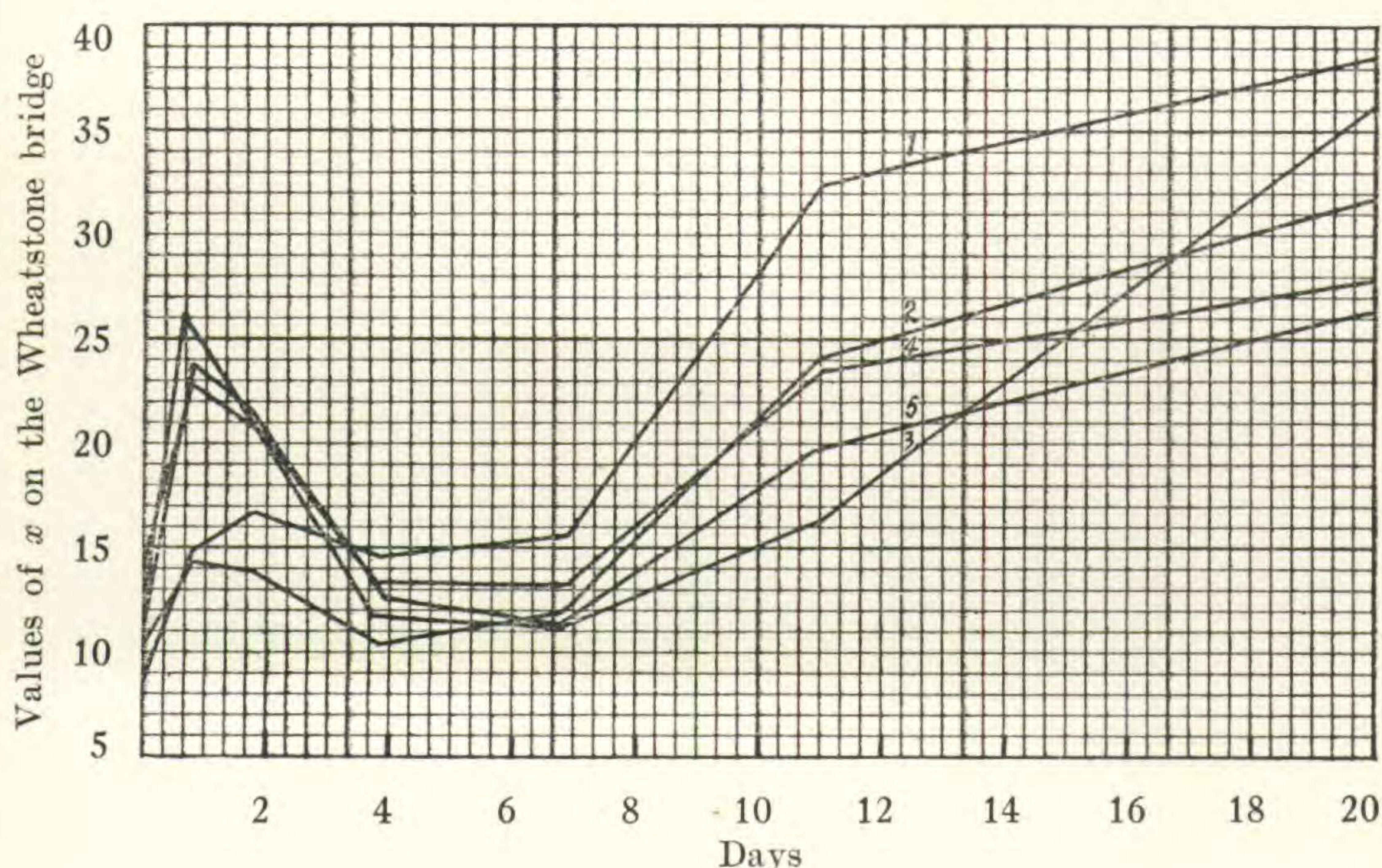


Fig. 15. Conductivity curves of cultures (series 16) in distilled water subsequent to treatment, as follows: No. 1, N/20 $MgCl_2$, 30 minutes; No. 2, N/20 $CaCl_2$, 30 minutes; No. 3, N/20 $MgCl_2$ plus N/20 $CaCl_2$, 30 minutes; No. 4, control—placed directly into distilled water; No. 5, N/20 $MgCl_2$, 4 hours. The cultures were 17 days old at the time of treatment. The first reading was taken in all cases after the roots had been in the distilled water exactly 30 minutes.

treatment with $MgCl_2$ is considered, and should no doubt be interpreted as an individual variation irrespective of treatment. In the case of No. 6, however, the curve for which represents the results of a 17-hour treatment with N/20 $MgCl_2$, we no doubt have a real effect clearly distinguished from the controls.

At the end of 20 days in distilled water following the treatment the tops of Nos. 1-10 were all in about the same condition, those of the treated plants showing no injury. Likewise the roots of Nos. 1-5 and 8-10 were practically normal, with no, or only very slight, flaccidity; those of No. 6, however, were brownish in color and somewhat flaccid, while those of

No. 7 were brownish only in spots, but were of about the same flaccidity as those of No. 6.

Having found that a treatment of 17 hours under the conditions indicated above was not sufficient to yield the most

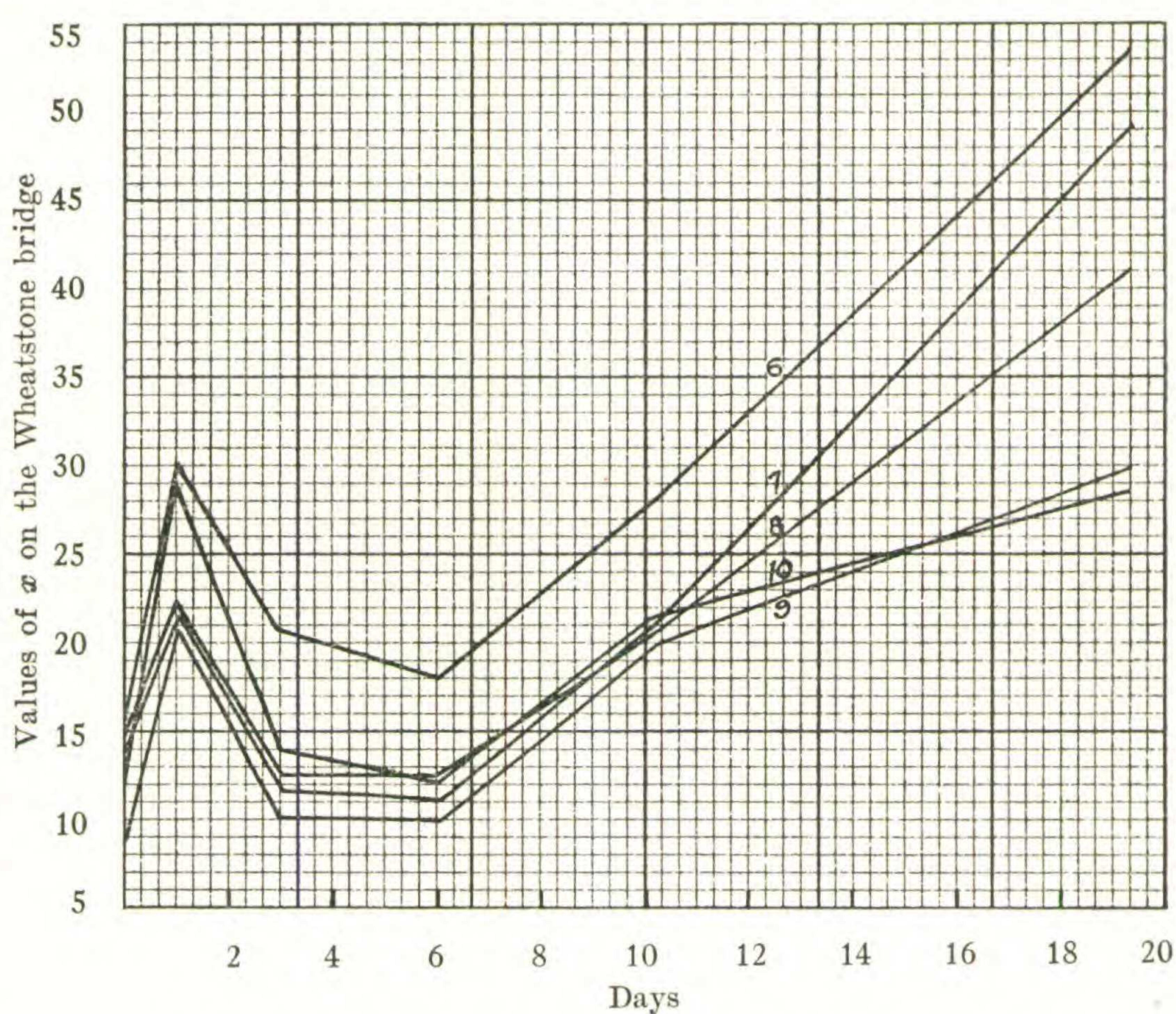


Fig. 16. Conductivity curves of cultures (series 16) in distilled water subsequent to treatment, as follows: No. 6, N/20 $MgCl_2$, 17 hours; No. 7, N/20 $CaCl_2$, 17 hours; No. 8, N/20 $MgCl_2$ plus N/20 $CaCl_2$, 17 hours; No. 9, control—distilled water, renewed after 17 hours; No. 10, control—distilled water, not renewed. The cultures were 17 days old at the time of treatment. The first readings were taken in all cases after the roots had been in the distilled water exactly 30 minutes. No. 10 remained in the full nutrient solution until the treated cultures were transferred (after the 17-hour period) from the respective solutions to distilled water.

positive results, it was decided to try stronger concentrations and longer periods. Figure 17 shows the conductivity curves after a period of treatment extending 75 hours. Some interesting results were obtained. N/10 $MgCl_2$ gave the highest readings, closely followed by N/10 $MgCl_2$ plus N/10 $CaCl_2$; the N/20 $MgCl_2$ plus N/20 $CaCl_2$ curve is very similar to that

obtained from N/10 $MgCl_2$ plus N/20 $CaCl_2$, while the N/10 $MgCl_2$ plus N/100 $CaCl_2$ causes a rise higher than that in the two curves just mentioned after the fifth day. It was unexpected that N/20 $CaCl_2$ should exceed N/20 $MgCl_2$ in its

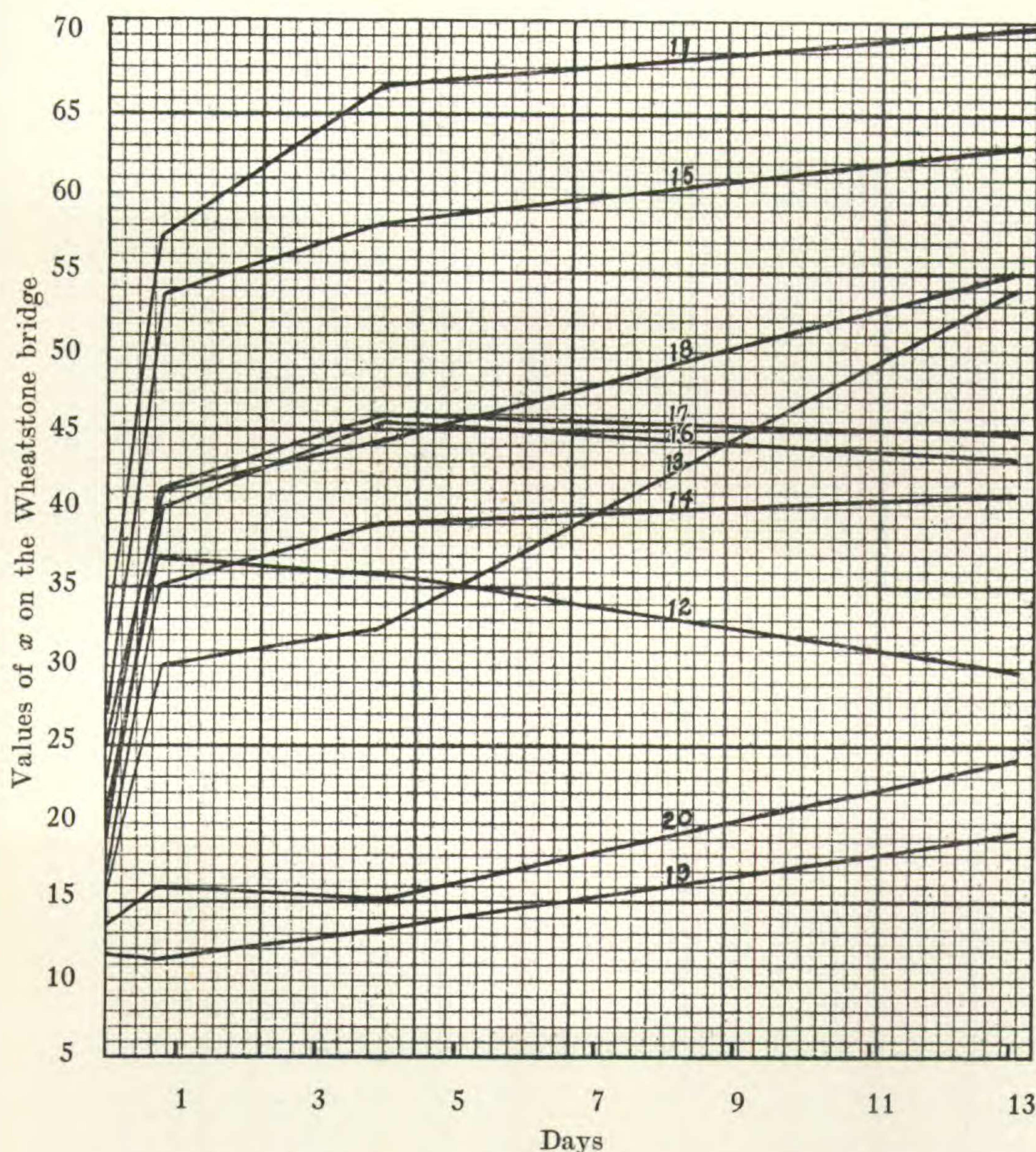


Fig. 17. Conductivity curves of cultures (series 16) in distilled water subsequent to treatment, as follows: No. 11, N/10 $MgCl_2$, 75 hours; No. 12, N/20 $MgCl_2$, 75 hours; No. 13, N/10 $CaCl_2$, 75 hours; No. 14, N/20 $CaCl_2$, 75 hours; No. 15, N/10 $MgCl_2$ plus N/10 $CaCl_2$, 75 hours; No. 16, N/20 $MgCl_2$ plus N/20 $CaCl_2$, 75 hours; No. 17, N/10 $MgCl_2$ plus N/100 $CaCl_2$, 75 hours; No. 18, N/10 $MgCl_2$ plus N/100 $CaCl_2$, 75 hours; No. 19, control—distilled water, renewed after 75 hours; No. 20, control—distilled water, not renewed. The plants were 21 days old when treated. The first reading was taken after the roots had been in the distilled water exactly 30 minutes (in No. 20, 75 hours). Nos. 19 and 20 were placed in distilled water at the same time that the cultures to be treated were placed in their respective solutions.

effect on exosmosis and that the conductivity curve resulting from treatment with N/10 CaCl_2 should rise so high at the end.

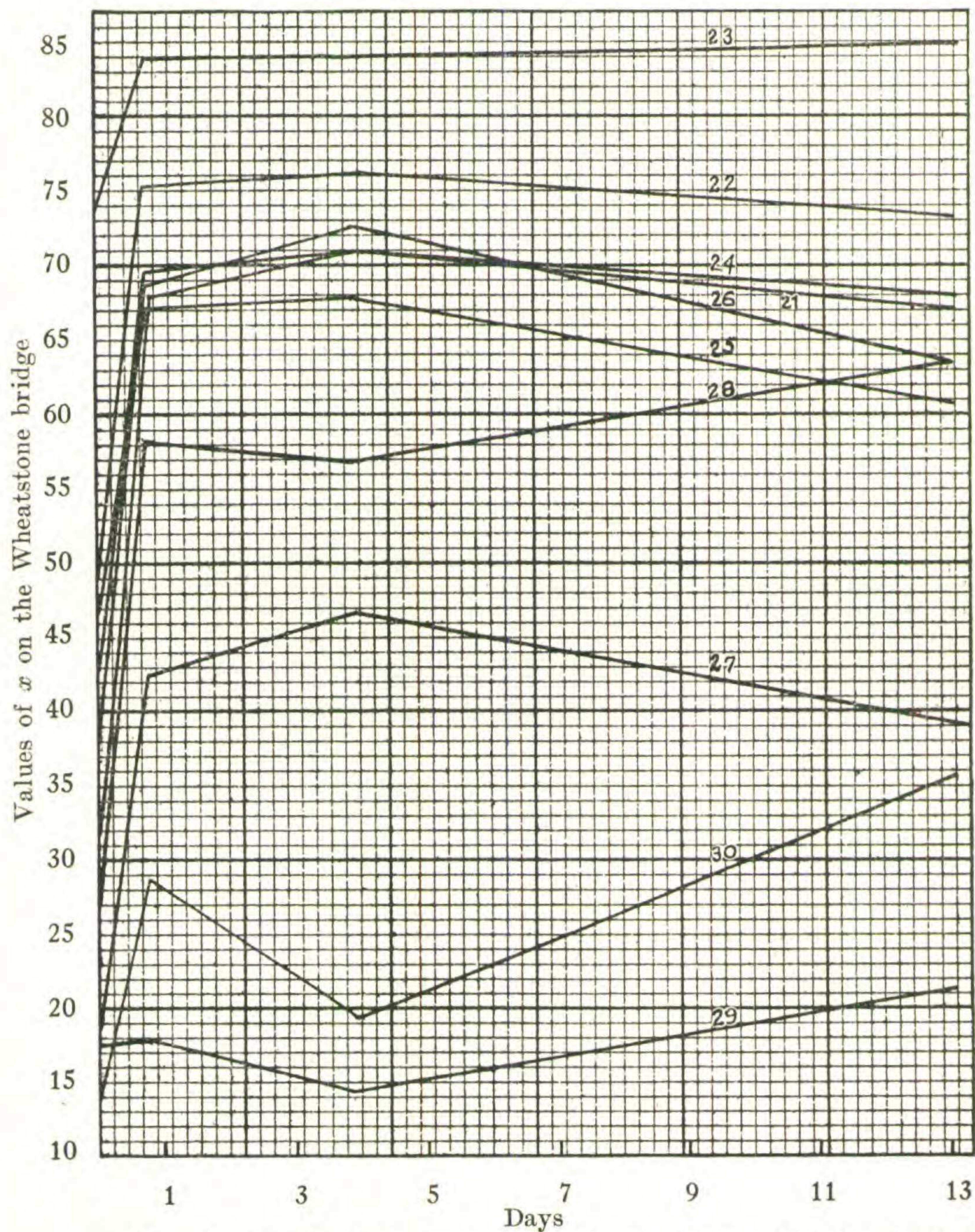


Fig. 18. Conductivity curves of cultures (series 16) in distilled water subsequent to treatment, as follows: No. 21, N/10 NaCl , 75 hours; No. 22, N/10 KCl , 75 hours; No. 23, N/10 NaCl plus N/10 KCl , 75 hours; No. 24, N/20 NaCl plus N/20 KCl , 75 hours; No. 25, N/10 NaCl plus N/10 CaCl_2 , 75 hours; No. 26, N/10 KCl plus N/10 CaCl_2 , 75 hours; No. 27, N/20 NaCl , 75 hours; No. 28, N/20 KCl , 75 hours; No. 29, control—distilled water, not renewed; No. 30, control—distilled water, not renewed. The plants were 21 days old when treated. The first reading was taken after the roots had been in the distilled water 30 minutes (in No. 29, 75 hours). Culture 30 was placed in distilled water at the end of the 75-hour period, having been in full nutrient solution up to that time.

At the end of 13 days in distilled water following the treatment, the tops of Nos. 11-20 were of the same appearance throughout, i. e., normal. The roots were also practically normal in the case of Nos. 12-20, except for a brownish color on those of Nos. 12, 13, 15, and 16-18, being especially evident in the case of No. 15. In addition to being brown, however, the roots of No. 11 were considerably flaccid.

Figure 18 shows similar relations for NaCl, KCl, and CaCl₂. It is seen that KCl is more effective than NaCl in causing exosmosis. Far from ameliorating the exosmotic condition, the treatment with combined NaCl and KCl likewise yields high conductivity readings of the medium, the N/10 concentration of each combined giving the highest. It can not be argued that this effect is due solely to the osmotic pressures of the solutions of the agents in question, for if that were the case we should expect more comparable results on the basis of the osmotic effects of the various solutions at the concentrations used. There is a reduction in the effect when the NaCl and KCl used singly are reduced to concentrations of N/20.

The condition of the plants 16 days after first applying the treatment, or 13 days after being in distilled water, is shown in table IX, from which it is evident that there was great exosmosis with but little or no visible effect accompanying it.

TABLE IX
CONDITION OF PLANTS TREATED WITH VARIOUS SALTS FOR
DIFFERENT PERIODS OF TIME

Culture no.	Condition of tops	Condition of roots
21 and 22	Normal	Slightly brown and very slightly flaccid
23	Dead—badly wilted at end of treatment	Very limp and flaccid and brownish
24-28	Normal	Very slightly brownish but practically normal
29 and 30	Normal	Practically normal

That osmotic effects play practically no part in the phenomenon under consideration is indicated from the results of Loeb ('03) on *Gammarus* and those of True ('14) on *Lupinus* seedlings. The writer also performed experimental work to

determine this point. Solutions of pure saccharose of varying concentrations were used and the effects produced by the same during a period of 24 hours as compared with pure distilled water, measured by the determined conductivity of the medium both during the 24 hours and after (when the plants which had been in the sugar solutions were also placed in distilled water). These results are given in table x. As there seen, no differences were obtained from the different concentrations.

TABLE X
EXOSMOSIS FROM THE ROOTS OF PLANTS IN SUGAR SOLUTIONS AND
DISTILLED WATER *

Time of readings	Culture 1 1.28% sac- charose sol'n. 24 hours followed by dist. H ₂ O	Culture 2 2.56% sac- charose sol'n. 24 hours followed by dist. H ₂ O	Culture 3 5.13% sac- charose sol'n. 24 hours followed by dist. H ₂ O	Culture 4 control dist. H ₂ O throughout changed every day conductivity readings
Conductivity readings of the sugar solutions:				
Before roots placed in the solution.....	9.4	10.6	10.9	10.9
After 10 hrs.....	28.0	19.7	30.8	32.9
After 24 hrs.....	28.9	18.3	29.0	32.9
Increase over original sol'n. during 24 hrs ...	19.5	7.7	18.1	22.0
Conductivity readings of the distilled water:				
After $\frac{1}{2}$ hr.....	9.4	8.6	8.8	8.3
After 23 hrs.....	10.5	11.5	11.0	10.4
After 48 hrs.....	10.9	11.3	10.2	10.0
Increase over dist. H ₂ O the first half hour †...	3.4	2.6	2.8	2.3
Increase over dist. H ₂ O during 48 hours †....	4.9	5.3	4.2	4.0

* All readings represent values of x on the Wheatstone bridge, the resistance in the box being 9,110 ohms.

† The average reading of the distilled water before placing roots in it was approximately 6.0.

In table xi are shown the effects produced by salts alone as well as by salts plus anesthetics in weak concentrations. It was desired to use approximately the same concentrations of anesthetics as indicated by the work of Lillie ('12), Osterhout ('13), and others. The conductivity of the water containing the anesthetics was not determined after the 53-hour treatment and hence the resulting exosmosis during that interval was not ascertained. But from other experiments on

the effect of anesthetics in solution we have seen that the exosmosis is rapid and considerable during the first day or so and then remains stationary, i. e., the curve becomes horizontal.

TABLE XI

EFFECTS OF SALT SOLUTIONS USED SINGLY AND COMBINED WITH ANESTHETICS UPON THE EXOSMOSIS FROM THE ROOTS OF PLANTS
(PLANTS 40 DAYS OLD WHEN TREATED)

Culture no.	Treatment	Conductivity*				
		Readings		Increase over dist. H ₂ O**		
		After $\frac{1}{2}$ hr.	After 42 hrs.	1st $\frac{1}{2}$ hr.	Next 41 $\frac{1}{2}$ hrs.	Total in 42 hrs.
31	N/10 MgCl ₂ , 53 hrs.....	35.4	57.1	29.4	21.7	51.1
32	N/10 NaCl, 53 hrs.....	34.4	60.9	28.4	26.5	54.9
33	N/10 KCl, 53 hrs.....	40.8	63.0	34.8	22.2	57.0
34	0.7% ether in H ₂ O, 53 hrs.....	10.0	11.6	4.0	1.6	5.6
35	0.7% CHCl ₃ in H ₂ O, 53 hrs.....	12.1	36.3	6.1	24.2	30.3
36	0.7% benzol in H ₂ O, 53 hrs.....	11.3	17.7	5.3	6.4	11.7
37	N/10 MgCl ₂ and 0.7% ether, 53 hrs.....	41.2	64.7	35.2	23.5	58.7
38	N/10 MgCl ₂ and 0.7% CHCl ₃ , 53 hrs.....	45.5	57.2	39.5	11.7	51.2
39	N/10 MgCl ₂ and 0.7% benzol, 53 hrs.....	44.0	49.4	38.0	5.4	43.4
40	N/10 NaCl and 0.7% ether, 53 hrs.....	37.4	59.5	31.4	22.1	53.5
41	N/10 NaCl and 0.7% CHCl ₃ , 53 hrs.....	47.5	62.4	41.5	14.9	56.4
42	N/10 NaCl and 0.7% benzol, 53 hrs.....	49.5	56.0	43.5	6.5	50.0
43	N/10 KCl and 0.7% ether, 53 hrs.....	50.2	76.0	44.2	25.8	70.0
44	N/10 KCl and 0.7% CHCl ₃ , 53 hrs.....	51.7	65.4	45.7	13.7	59.4
45	N/10 KCl and 0.7% benzol, 53 hrs.....	49.2	55.8	43.2	6.6	49.8
46 and 47	Control (dist. H ₂ O renewed after 53 hrs.)...	10.9	11.9	4.9	1.0	5.9
48	Control (dist. H ₂ O not renewed).....	15.2†	15.5‡	9.2§	.3	9.5
49 and 50	Control (full nutr. until Nos. 31-45 were placed in dist. H ₂ O).....	12.5	39.0	6.5	26.5	33.0

* All readings represent values of x on the Wheatstone bridge with a resistance in the box of 9,110 ohms.

** The average reading of the distilled water before placing roots in it was approximately 6.0.

† After 53 hours. ‡ After 95 hours. § In 53 hours. || In 95 hours.

zontal. We can therefore safely infer that such was the case here, except possibly in the ether-treated cultures in which the roots and tops showed no effect whatever from the treat-

ment. We can thus account for the fact that the increase in the conductivity of the medium in Nos. 34, 35, and 36, was so slight and so similar to that given by the controls. It will be seen that the anesthetics did not antagonize the salts so far as exosmosis of electrolytes is concerned. The condition of the plants after the 53-hour treatment is shown in table XII.

TABLE XII
CONDITION OF PLANTS FIFTY-THREE HOURS AFTER TREATMENT WITH
SOLUTIONS OF SALTS AND ANESTHETICS

Culture no.	Condition of tops	Condition of roots
31	Normal.....	Yellowish brown, somewhat flaccid
32	Practically normal.....	Slightly yellow, practically normal
33	Subnormal, drying considerably.....	Slightly yellow, practically normal
34	Normal.....	Practically normal
35	Normal.....	White, but considerably flaccid
36	Almost normal.....	Very flaccid
37	Practically normal	Yellowish and considerably flaccid
38	Almost normal.....	Somewhat flaccid
39	Drying considerably.....	Very flaccid
40	Normal.....	Almost normal
41	Drying somewhat.....	Considerably flaccid
42	Drying considerably.....	Very flaccid
43	Practically normal.....	Practically normal
44	Drying considerably.....	Considerably flaccid
45	Drying badly.....	Very flaccid
46-50	Normal.....	Practically normal

The concentration of the anesthetics used in the above experiments was near the boundary which would just produce exosmosis. To eliminate such action entirely when these substances were used alone, therefore, the concentrations used were reduced to a point below that at which they cause exosmosis to any appreciable extent, if at all. The results of that series are given in table XIII, where we see again no indications that there is any decreasing effect by the anesthetics on the exosmosis induced by salts. On the contrary, the combined salt and anesthetic cause a greater exosmosis than the salt alone.

As measured by the resulting growth of roots, Hibbard ('13) found an antagonistic action between CuSO_4 and chloral hydrate. To determine if such action would also hold true in

the case of exosmosis, an experiment was set up, the results of which are given in table XIV. As there seen, there was no decrease in the exosmosis caused by either substance when the two were combined.

TABLE XIII

EFFECTS OF SALT SOLUTIONS USED SINGLY AND COMBINED WITH ANESTHETICS ON THE EXOSMOSIS FROM THE ROOTS OF TREATED PLANTS*

Culture no.	Treatment	Conductivity					
		Readings	Increase over dist. H ₂ O				
			After $\frac{1}{2}$ hr.	After 41 hrs.	1st $\frac{1}{2}$ hr.	Next $40\frac{1}{2}$ hrs.	Total in 41 hrs.
1	1/8 saturated CHCl ₃ in H ₂ O, 44 hrs.....	8.5†	11.0	2.5‡	2.5	5.0	
2	M/200 chloral hydrate, 44 hrs.....	13.4†	18.9	7.4‡	5.5	12.9	
3	N/10 NaCl, 44 hrs.....	38.4†	59.3	32.4‡	20.9	53.3	
4	N/10 KCl, 46 hrs.....	53.6	76.2	47.6	22.6	70.2	
5	1/8 saturated CHCl ₃ in H ₂ O & N/10 NaCl, 44 hrs.....	46.2	61.6	40.2	15.4	55.6	
6	1/8 saturated CHCl ₃ & N/10 KCl, 46 hrs.....	47.2	76.5	41.2	29.3	70.5	
7	M/200 chloral hydrate & N/10 NaCl, 46 hrs..	49.1	79.3	43.1	30.2	73.3	
8	M/200 chloral hydrate & N/10 KCl, 46 hrs ..	60.5	84.2	54.5	23.7	78.2	
9	N/10 NaCl & N/10 KCl 46 hrs.....	70.7	82.7	64.7	12.0	76.7	
10	N/20 NaCl & N/20 KCl, 46 hrs.....	41.8	71.7	35.8	29.9	65.7	
11	Control (dist. H ₂ O renewed every 2 days)..	11.4	10.8	5.4	-.6	4.8	
12	Control (dist. H ₂ O not renewed).....	16.7	25.0	10.7	8.3	19.0	

* All readings represent values of x on the Wheatstone bridge with a resistance in the box of 9,110 ohms.

† Reading taken after 50 minutes. ‡ Increase in first 50 minutes.

|| The average reading of the distilled water before placing roots in it was approximately 6.0.

Merely to get a basis of comparison between the effects produced by the various agents above mentioned and acid and alkali in certain concentrations, plants were placed in solutions of KOH and H₂SO₄ of approximately the limiting concentrations for root growth, as found by Kahlenberg and True ('96). Instead of excretion being greater than absorption the reverse was found to be true during the period the plants remained in the solutions. The plants, to all external appearances, were not affected adversely in the least, and when later

placed in distilled water gave practically no greater exosmosis than the control. With stronger concentrations a marked effect would undoubtedly be produced. The results obtained are given in table xv. Another point worthy of note in this

TABLE XIV
EFFECTS OF COPPER SULPHATE AND CHLORAL HYDRATE USED SINGLY AND COMBINED UPON THE EXOSMOSIS FROM THE ROOTS OF PLANTS

Cult. no.	Treatment	SPECIFIC CONDUCTIVITY OF THE SOL'NS.*			VALUES OF x , † OR BRIDGE READINGS OF THE DISTILLED WATER				Total increase in 63 hrs. ‡‡
		Before roots in the sol'n.	After 27 hrs. in the sol'n.	Increase in 27 hrs.	After $\frac{1}{2}$ hr. in the H_2O	After 63 hrs. in the H_2O	Increase the 1st $\frac{1}{2}$ hr. ‡‡	Increase the next $62\frac{1}{2}$ hrs.	
1	M/10,000 $CuSO_4$, 28 hrs.....	2.92	16.68	13.76	19.7	55.4	13.7	35.7	49.4
2	M/100 $CuSO_4$, 27 hrs.....	142.40	151.10	8.70	22.0	22.4	16.0	.4	16.4
3	M/8,000 chloral hydrate, re- maining to end of exp.....	.35	1.16	.81	20.6‡	13.1**	14.6‡	-7.5	7.1**
4	M/100 chloral hy- drate, 26 hrs...	.37	3.04	2.67	14.8	16.8	8.8	2.0	10.8
5	M/10,000 $CuSO_4$ a n d M/8,000 chloral hydrate, 26 hrs.....								
6	M/100 $CuSO_4$ and M/100 chloral hydrate, 26 hrs.	2.82	19.77	16.95	15.0	53.3	9.0	38.3	47.3
7	Control (dist. H_2O , changed every 4 days).....	82.69	95.25	12.56	17.2	20.2	11.2	3.0	14.2
8	Control (dist. H_2O , not changed).....	.32	.79	.47	15.0	12.9	9.0‡	-2.1	6.9
					14.8	10.7	8.8	-4.1	4.7

* The values given are to be multiplied by 10^{-5} to obtain specific conductivity values.

† Resistance in box 9,110 ohms.

‡ After 26 hours in the solution.

§ The first 26 hours.

|| After 26 hours in distilled H_2O .

‡‡ The average reading of the distilled water before placing roots in it was ap-
proximately 6.0.

** After 89 hours in the solution.

†† After 89 hours in the water.

connection and seen in table xv is the additional verification of the fact that the rinsing method used throughout this investigation was effective and that no electrolytes were carried

over on the roots from the full nutrient solutions, salt solutions, and other media to the distilled water, at least not in sufficient quantity to affect the validity of the results in any way. Although the conductivity of the acid and alkaline media was very high, it is seen by reference to table xv that after rinsing the roots in the usual manner and transferring the cultures to distilled water the readings were very low, thus showing that practically no electrolytes were carried over on the roots.

TABLE XV
CONDUCTIVITY READINGS OF THE CULTURE MEDIA OF PLANTS IN KOH, H₂SO₄, AND LATER IN DISTILLED WATER

Cult. no.	Treatment	SPECIFIC CONDUCTIVITY* OF THE SOL'NS.				VALUES OF x,† OR BRIDGE READINGS OF THE DISTILLED WATER				
		Before roots in the sol'n.	After 22 hrs. in the sol'n.	After 47 hrs. in the sol'n.	Increase in 47 hrs.	After $\frac{1}{2}$ hr. in the H ₂ O	After 50 hrs. in the H ₂ O	Increase the 1st $\frac{1}{2}$ hr.††	Increase the next $49\frac{1}{2}$ hrs.	Total increase in the 50 hrs.††
1	N/12,800 H ₂ SO ₄ , 47 hrs.....	2.39	1.50	1.03	-1.36	10.8	10.5	4.8	-.3	4.5
2	N/6,400 H ₂ SO ₄ , 47 hrs.....	5.62	2.53	1.17	-4.45	8.7	9.6	2.7	.9	3.6
3	N/400 KOH, 47 hrs.....	42.02	20.09	16.39	-25.63	11.5	8.7	5.5	-2.8	2.7
4	N/200 KOH, 47 hrs.....	58.50	27.98	24.16	-34.34	11.2	10.5	5.2	-.7	4.5
5	Control (dist. H ₂ O, not changed)...	.98	2.56	1.32	.34	22.8‡	17.8	16.8‡	-5.0	11.8

* The values given are to be multiplied by 10⁻⁵.

† The resistance in the box was 9,110 ohms.

‡ After being in distilled water 47 hours.

†† The average reading of the distilled water before placing roots in it was approximately 6.0.

** After 97 hours.

IX. GENERAL DISCUSSION

In the foregoing experiments we have been able to note the exosmosis of electrolytes following different treatments. As compared with the controls we have seen marked excretions in some cases and slight or no exosmosis in excess of that in the controls in others. In the normal untreated cultures, or controls, we have seen that there is almost universally a slight exosmosis from the roots into the distilled water for about 24

hours or so, and then in most cases there is a decline in the conductivity curve to a point approaching the original position, after which there may or may not be a gradual incline, depending, probably, on various factors.

It might be well briefly to consider some theoretical aspects of the subject, especially in regard to the causal agencies effecting the increased exosmosis of the treated cultures. The mere transfer of a culture from a full nutrient solution to distilled water is not in itself sufficient to account for the effects produced, as we have seen that osmotic effects play little or no rôle in this connection, a conclusion in harmony with the findings of Loeb ('03) and of True ('14). To what then is the exosmosis due? Can it all be laid at the door of cell cytolysis? What influence has an alteration of the plasma membrane?

In any case, we are dealing with the effect of physical and chemical factors upon the plant cell. For our purpose here it is not considered necessary to enter upon a discussion of the various ideas regarding the details of the structure of the cell and its limiting membrane, or the work and theories of the different investigators on both the animal and plant side concerning the permeability of the plasma membrane. Yet in passing, it may be well to mention Overton's theory regarding the lipoid nature of the plasma membrane, Nathansohn's idea of a mosaic structure of the same, Czapek's experiments indicating the presence of neutral fats in the membrane, Lepeschkin's view that the plasma membrane is a continuous film (some of the work of the last two investigators being summarized by Blackman, '12), and Kite's work on the structure of protoplasm, and also make note of the recent work of Cranmer ('14) on the lipoid content of the cell wall.

The effect of the two physical factors, heat and cold, may undoubtedly be considered as resulting in a complete or incipient disorganization of the cell, depending upon the duration of exposure, and a consequent escape of some of the contents into the surrounding medium.

In the case of the various chemical factors or agents used the matter is probably not so simple or so easily disposed of. However, a conception that would fulfill the requirements

theoretically and also accord with the experimental results would seem to be based on the specificity of chemical reaction. The cell, with its complex aggregation of chemical substances, may be considered as interacting with the substance employed, be it anesthetic, toxic agent, salt solution, or other chemical. It may be assumed that each substance has a greater affinity (if we may use that tabooed chemical term) for a particular component of the cell than for other constituents and hence reacts accordingly. This was exemplified by the striking comparison between the effect produced by anesthetics in certain concentrations and that produced by the KCl or NaCl solution. The exosmosis, it is true, was considerable in both cases, but the resulting appearance of the roots was markedly different, the anesthetics causing indications of flaccidity, while the roots exhibiting quite as much exosmosis in the salt solutions, remained practically normal. If we assume that the anesthetic acted upon the colloidal matrix or gel portion of the cell and thus more or less destroyed its organization, while the salts reacted with the substances in the sol condition and left the matrix more or less intact, we would seem to have a basis for explaining the differences observed.

Anesthesia has been considered by Lillie, Osterhout, and others to be essentially a reversible process, provided that the concentration of the anesthetic was not sufficient to be toxic. The experimental work reported herewith, however, on the excretion of electrolytes induced by various anesthetics does not seem to substantiate that view. If the concentration of the anesthetics employed was below a certain point there was no observable effect whatsoever. By increasing the concentration the critical point was attained when excretion began, and as the concentration of the anesthetic was further increased, or as the period of application was lengthened, excretion likewise increased. The excretion process induced by anesthetics therefore conformed in every way to an irreversible chemical reaction. In Osterhout's conductivity measurements of tissue, secondary agglutinization phenomena may possibly have entered in to give the observed effects, and thus have masked the real chemical reaction. Recovery of organ-

isms after anesthetic treatment has also been considered by some as evidence indicating the reversibility of the anesthetic action. If such be viewed from the standpoint of chemical reactions, however, the mere fact of recovery of the organism to a normal condition following the application of anesthetics would not seem to be sufficient justification for concluding that the chemical reaction which initiated the effect is a reversible one, especially when one considers the manifold activities of the cell and the wonderful recuperative powers possessed by organisms, these no doubt involving numerous reactions. Hence the writer is inclined to the belief that an irreversible chemical reaction was at the basis of the phenomena observed as a result of the treatment of the plant with anesthetics and the consequent exosmosis of substances contained in the cell, and that any alteration of the plasma membrane resulting in changed permeability finds its best explanation on the basis of actual chemical reactions.

It is further believed that the results obtained by antagonistic pairs of salts and by single salts are also to be explained, as far as resulting exosmosis is concerned, in the specificity of the action of each. The method employed herein gives a delicate register of such action and is considered to be especially desirable because in it growth phenomena, with their resulting complex nutritive relations, may be left out of consideration. That the high conductivity readings in the case of the salts and certain other electrolytes was not due to insufficiency of the washing before the roots were placed in the distilled water was abundantly proved in various ways.

In regard to the method of experimentation employed in the work here reported, mention may well be made of its adaptability for delicate determinations pertaining to the relative toxicity of different substances. In the past such determinations have been made by means of growth measurements. It would seem that in this method we have, in some respects, a more rapid and satisfactory procedure for such work.

X. SUMMARY AND CONCLUSIONS

A brief historical review is given of the subject of excretion

from plant roots, exosmosis from living cells, and of excretion from leaves and other tissues.

The methods of experimentation are described.

A theoretical discussion is given of the various aspects of the subject.

The following are some of the experimental results obtained:

(a) Pea seedlings grew better in distilled water in which exosmosis from the previously treated plants of the first crop had occurred than in fresh distilled water, or in distilled water in which untreated plants had been grown.

(b) Peas and horse beans did not do as well in distilled water in which pea seedlings had already grown for 21 days as in fresh distilled water.

(c) Abundant exosmosis may occur from treated plants, even though the roots remain entirely normal in appearance. When the tops were badly affected and the roots remained normal, abundant exosmosis also occurred and the indications pointed in some cases to a downward flow of substances into the roots and out into the aqueous medium. No conclusive proof of this was obtained, however.

(d) Anesthetic vapors cause marked exosmosis upon considerable exposure of the plants to them, but there is none if the exposure be short. The interval required to initiate exosmosis was accurately determined. The order of effectiveness of the vapors tried is, ether, least; illuminating gas, more; and chloroform, most.

(e) The time limits for the exposure of plants to extremes of temperature in relation to exosmosis were determined. Comparison was also made between the effect of dry and moist heat.

(f) The exosmosis curves for various organic compounds were found. In general, at the concentrations used, marked excretion was produced.

(g) The effects of single salts, salts in pairs, and salts plus anesthetics in solution were ascertained as regards the exosmosis produced upon the plants in such solutions. Antagonistic relations in the sense of one substance decreasing the

exosmotic effect produced by another substance were found not to hold in the cases tried and under the conditions of the experiment.

It is with pleasure that the writer acknowledges his indebtedness to Dr. B. M. Duggar for numerous helpful suggestions in the prosecution of this work, and to Mrs. Amy Lyman Merrill for valuable assistance in the calculations involved and in the plotting of the curves; also to Dr. J. R. Schramm, who kindly aided in the tedious work of preparing the manuscript for the printer.

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